# (Check) Ethnic Fermented Foods

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# 14 Ethnic Fermented Foods and Beverages of Indonesia

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## 14.1 Introduction

Indonesia is the largest archipelago in the world extending some 2000 km from north to south and more than 5000 km from east to west and consists of 17,508 islands, about 6000 of which are inhabited, scattered over both sides of the equator (Fig. 14.1). The archipelago stretches over more than one tenth of the equator between Southeast Asia and Australia. The largest islands are the Kalimantan, Sumatra, Papua, Sulawesi, and Java. Indonesia lies between latitudes 11°S and 6°N and longitudes 95°E and 141°E and consists of islands (CIA 2015).

The temperature ranges between 16 and 35 °C with humidity ranging from 60 % to 98 %. There are two seasons, the rainy monsoon season which usually lasts from November through May, with the heaviest rainfall from November through March, followed by the dry season which is driest between June and September. Rainfall varies throughout Indonesia, averaging 706 mm (28 in.) yearly.

Indonesia has a population of 255,993,674 people (estimated per July 2015) and is the fifth most populous nation in the world after China, India, EU, and the United States, the majority of which are of Malay extraction. The remainder of the natives is Melanesian (in Papua and the eastern islands). There are ethnic Chinese, Indians, and Arabs concentrated mostly in urban areas throughout the archipelago. There are about 300 ethnic groups, each with cultural identities developed over centuries and influenced by Indian, Arabic, Chinese, and European sources, and 742 different languages and dialects. Major ethnic groups are Javanese (45 %), Sundanese (14 %), Madurese (7.5 %), Coastal Malays (7.5 %), and others (26 %) (Expat website Association 2015).

The agricultural sector of Indonesia comprises large plantations (both state owned and private) that tend to focus on commodities which are important export products (palm oil and rubber) and smallholder production modes that focus on rice, soybeans, corn, fruits, and vegetables. According to FAO of the United Nations (2015), the top 11 products of Indonesia in 2012 include paddy rice, palm oil, rubber, chicken, cassava, maize, coconuts, banana, palm kernels, mango, and mangosteens.

Rice is a staple in Indonesia, except in Papua and Maluku where people sustain themselves with sago, which is a type of tapioca; sweet potatoes; and cassava. Indonesian cuisine is as varied as its culture, and the food in Indonesia is as diverse as its geography with the influences from China, Europe, and even India, rich in flavors; soy-based dishes, such as variations of tofu (*tahu*) and *tempe*, are also very popular (Table 14.1).

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**Fig. 14.1** Map of Indonesia

Fermentation is one of the oldest and most economic methods in preserving the quality and safety of foods; it not only prolongs the shelf life but also reduces volume, shortens cooking times, provides better nutritional bioavailability, enhances flavor and aroma, and can be considered as a functional food that exerts health-promoting benefits (Tamang 2015). A rich variety of indigenous traditional fermented foods involving yeast, mold, bacteria, and their combination, owned by each area, are an important part of the culture, identity, and heritage and have certain distinct sensory characteristics as a result of metabolite accumulation produced by microbes involved, contributing to flavor, texture, and aroma. Traditional fermented foods and beverages are also considered as important part of diet due to its high nutritive value, digestibility, and reduced antinutrient compounds. Fermentation may assist in the detoxification of certain undesirable compounds such as toxin and antinutrients which may be present in raw foods, such as phytates, polyphenols, and tannins (Sharma and Kapoor 1996). The manufacture of fermented foods uses diverse raw materials as substrates, such as cereals, legumes, tubers, fruits, vegetables, animal such as meat and milk, and marine sources; many of them are made only on home scale in traditional methods of preparation passed on from generation to generation using relatively simple equipment at very low cost with insufficient hygienic precautions (Surono and Hosono 1994a, b).

Most of the traditional food fermentations are conducted by natural, spontaneous fermentation involving mixed beneficial microbes from staple ingredients and environmental surrounding as home industry. As a consequence, pure and single culture will not be involved; natural contamination and inconsistent quality of the product may occur due to lack of sterility and the use of natural fermentation (Nout and Sarkar 1999). Based on the substrate used, fermented foods and beverages can be classified into:

Fermented grain, cereals, and legume foods
Fermented fruits and vegetable products
Fermented milk products
Fermented fish and meat products
Fermented roots and tuber products

**Table 14.1** Ethnic fermented foods and beverages of Indonesia

| Foods | Substrates | Nature and uses | Microorganisms | Regions of consumption in Indonesia | References |
|---|---|---|---|---|---|
| *Tempe* | Soybeans | Side dish | *Rz. oligosporus* | All regions | Nout et al. 2000 |
| *Oncom* | Soybean, peanut | Side dish | *N. sitophila*, *Rz. oligosporus* | West Java | Sastraatmadja et al. (2002), Hoo (1986), Afifah et al. 2014, Sulchan and Nur (2007), and Sumi and Yatagai (2006) |
| *Gembus* | Soybean | Side dish | *Rz. oligosporus* | Central Java | Kuswanto (2004), Sulchan and Rukmi (2007), Sulchan and Nur (2007). and Fatimah (1998) |
| *Kecap* | Soybean | Condiment | *A. oryzae*, *A. sojae*, *Rz. oryzae*, *Rz. oligosporus* | All regions | Steinkraus (1995) and Judoamidjojo (1986) |
| *Acar* | Vegetables | Condiment | *Lb. plantarum* | All regions | Lennox and Efiuvwere (2013) |
| *Sayur asin* | Vegetables | Condiment | *Lb. plantarum*, *Leu. mesenteroides*, *Lb. confusus*, *Lb. curvatus*, *P. pentosaceus* | West Java | Sulistiani et al. (2014) and Puspito and Fleet (1985) |
| *Tauco* | Soybean | Condiment | *R. oligosporus*, *Rz. oryzae*, *A. oryzae*, *Lb. delbrueckii*, *Hansenula* | West Java | Winarno et al. (1973) |
| *Tempoyak* | Flesh of durian (*Durio zibethinus*) | Condiment | *Ent. gallinarum* UP-9, *Ent. faecalis* UP-11, *Oenococcus*, *Leuconostoc*, *Enterococcus*, *Lactococcus*, *Pediococcus acidilactici*, *Lactobacillus*. *Leuconostoc* sp. | Sumatra | Wirawati (2002), Pato and Surono (2013), Widowati et al. (2013), and Yuliana and Garcia (2009) |
| *Mandai* | Inner part of *cempedak* or jackfruit | Condiment | *P. pentosaceus*, *Lb. plantarum*, *Lb. pentosus* | Kalimantan | Emmawati (2014), Rahayu (2003), (2010) |
| *Brem* | Cassava, glutinous rice | Snack, beverages | *Rz. oryzae*, *M. rouxii*, *A. oryzae*, *S. cerevisiae*, *Acetobacter aceti* | Central Java, Bali | Basuki (1977), Saono et al. (1984), and Aryanta (2000) |
| *Tuak* | Juice of plant | Beverages | *S. cerevisiae*, *C. tropicalis* | North Sumatra, Nusa Tenggara | Hermansyah et al. (2015) |
| *Dadih* | Buffalo milk | Beverage | *Lc. lactis* subsp. *lactis*, *Lb. brevis*, *Lb. plantarum*, *Lb. casei*, *Lb. paracasei*, and *Leu. mesenteroides* | West Sumatra | Imai et al. (1987) and Surono (2003a, b) |
| *Urutan* | Meat, pork | Side dish | *Lb. plantarum*, *Lb. farciminis*, and obligate heterofermentative lactobacilli *Lb. fermentum* and *Lb. hilgardii*. Besides, *P. acidilactici* and *P. pentosaceus* | Bali | Antara et al. (2002) and Aryanta (1998) |

(continued)

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| Foods | Substrates | Nature and uses | Microorganisms | Regions of consumption in Indonesia | References |
|---|---|---|---|---|---|
| *Peda* | Fish | Side dish | *Lb. plantarum*, *Lb. curvatus*, *Lb. murinus* and *Strep. thermophilus* | Java | Rahayu (2003) |
| *Terasi* | Fish and shrimp | Condiment | *Bacillus* sp. and *Pseudomonas* sp. | Sumatra, Java | Surono and Hosono (1994a) |
| *Telur asin* | Duck egg | Side dish | *Lb. plantarum*, *Lb. casei* subsp. *rhamnosus*, *Enterococcus gallinarum*, and *P. acidilactici* | All region | Suprapti (2002) and Saputra (2013) |
| *Tape* | Cassava, glutinous rice | Snack | *Rz. oryzae*, *M. rouxii*, *A. oryzae*, *S. cerevisiae*, *E. burtonii*, *H. anomala*, and *P. pentosaceus*. *Lb. plantarum* and *Lb. fermentum* | West Java, Central Java | Aryanta (1988) and Uchimura et al. (1998) |
| *Growol* | Cassava | Snack | *Coryneform*, *Streptococcus*, *Bacillus*, *Actinobacteria* *Lactobacillus*, and yeast | Yogyakarta | Suharni (1984) |
| *Gatot* | Cassava | Staple food | *P. pentosaceus*, *Saccharomyces* sp. *TR7*, *Lb. plantarum* 250 Mut7 FNCC | Yogyakarta | Ichsyani (2014) |

## 14.2 Traditional Fermented Foods of Indonesia

### 14.2.1 Fermented Grains/Legumes and Cereals

*Tempe*, *oncom*, *tauco*, and *kecap* are all Indonesian legumes and grain fermented foods. *Tempe* and *oncom* are solid fermented foods, tauco is in the form of paste or slurry, and *kecap* is a liquid fermented food. Historically, most traditional soy protein foods originated from China and were introduced later to other countries in the East and Southeast Asia (Smith 1963). *Tempe* is unique among major traditional soya foods, because it is the only fermented soya food product that did not originate from China or Japan (Shurtleff and Aoyagi 2007). Today, Japan leads to industrialization, technology development, equipment manufacture, and worldwide soybean-based food marketing.

## 14.3 History, Manufacture, Biochemical and Nutritional Value, and Socioeconomics of *Tempe*

### 14.3.1 History of *Tempe*

The word *tempe* appears to have originated in Central Java, Indonesia. It is not derived from Chinese (as other soy foods in Indonesia), and it does not start with the prefix *tau* or *tao* (as do *tauci*, *tauco*, *taugé*, *taujiong*, *tahu*, *takua*) (Astuti 1999). The earliest known *tempe* reference is found in the *Serat Centhini* manuscript and first cited in *History of Tempeh* (Shurtleff and Aoyagi 1979) and then in *The Book of Tempeh* (Shurtleff and Aoyagi 1985), so it is presumed that *tempe* existed in Java in the early 1600s. The *Serat Centhini* (the Centhini manuscript), a classic work of modern Javanese literature and a kind of encyclopedia, was probably written around 1815.

"Serat" means manuscript or work or tale. "Centhini" (also spelled "Centini") refers to a character in the book written in verse, and the information is given often very detailed on many different subjects – not just religion but also various aspects of Javanese culture and life. On one page the word *tempe* appears, indicating that tempe was produced in the early seventeenth century (Okada 1988).

Prinsen Geerligs (1896), a Dutchman, is the first to spell the word *tempeh* (with an "h" on the end) and also the first to name the *tempeh* mold as *Rhizopus oryzae*. Other authors from the Dutch use the spelling *témpé* (Gericke and Roorda 1875; Heyne 1913) or *tèmpé* (Vorderman 1902; Stahel 1946).

In 1905, Dr Kendo Saito of Tokyo Imperial University described that the main *tempeh* microorganism is *Rhizopus oligosporus* (Kendo 1905). *Tempe* was introduced to the Japanese by Dr Nakano Masahiro in 1958 and some published papers on *tempeh* were written by Japanese scientist (Nakano 1959; Ohta et al. 1964; Ohta 1965, 1971; Nakano 1967, Watanabe et al. 1971). Indonesians pronounce the word *tempe*, which is the correct spelling in Indonesian language. Van Veen (1962) reported that the attempt to introduce *tempeh* to Indian population by missionaries in Travancore, in Southern India in 1936, was not successful, since they did not have any interest in this unknown fermentation product.

### 14.3.2 Manufacturing Tempe, Biochemical and Nutritional Values

*Tempe* is legumes' fermentation with the aid of mold, *Rhizopus* sp. The hydrated, cooked, dehulled whole soybeans are fermented by *Rhizopus* sp. molds. It is a moist solid cake with a mild, pleasant taste. According to Steinkraus (1980), *tempe* is a single cell protein grown on edible substrate. *Tempe* fermentation is similar to cheese fermentation since the hydrolysis of protein and lipid occurs, flavor intensifies, and free amino acid is released (Steinkraus 1983). The traditional product is highly perishable and is usually consumed the day it is made. In industrial production, it can be preserved by drying or freezing (after blanching to inactivate the mold and its enzymes).

There are two distinct fermentation periods. The first occurs during soaking of the soybean and results in acidification by lactic acid bacterial fermentation. Second is fungal fermentation and results in mycelial growth and partial digestion of the enzymes from the mold (Steinkraus et al. 1960). In the preparation, the soybeans (*Glycine max*) are soaked overnight in three volumes of water containing 10 mL of 0.85 % lactic acid per liter of water or *Lb. plantarum*, a lactic acid-producing bacterium that can be added to the soak water in place of lactic acid. The soak water is acidified to about pH 5.0 to inhibit the growth of microorganisms which can cause spoilage, boiled, drained, cooled, and spread out on a tray, followed by mixing with a little molded *tempe* cake from a former batch or adding fermentation starter containing the spores of *Rz. oligosporus* (Fig. 14.4); Wang et al. (1975) recommended $10^6$ spores per 100 g cooked soybean for optimal fermentation, then wrapped with banana leaves, and kept overnight for about for 24–36 h at room temperature until the mass is bound by cottony mycelium of the mold into a solid white cake. *Tempe* is often produced in Indonesia using *Hibiscus tiliaceus* leaves, called *usar*. The undersides of the leaves are covered in downy hairs known technically as trichomes to which the spores of *Rz. oligosporus* can be found adhering. During fermentation, there is some biochemical reaction occurred involving some enzymes; hence, *tempe* is more digestible as compared to soybean (VanVeen and Schaefer 1950). The fermentation eliminates the beany flavor of raw soybeans and gives the product a bland but attractive flavor (Hesseltine and Wang 1967). Martinelli and Hesseltine (1964) introduced the use of plastic bags as containers for *tempe* fermentation, which is perforated to provide the moderate aeration necessary for mold growth without excessive sporulation, resulting in an attractive creamy, white fresh *tempe* cake (Figs. 14.2 and 14.3). This new idea and new technology is quickly transferred to *tempe* makers in Java and becomes widely used (Fig. 14.4 and 14.5).

**Fig. 14.2** *Tempe* in plastic bag

**Fig. 14.3** Fermented soybean cake, *Tempe*

**Fig. 14.4** Adding inoculums of *Rz. oligosporus* to the dehulled cooked soybeans (Source: Rumah *Tempe* Indonesia)

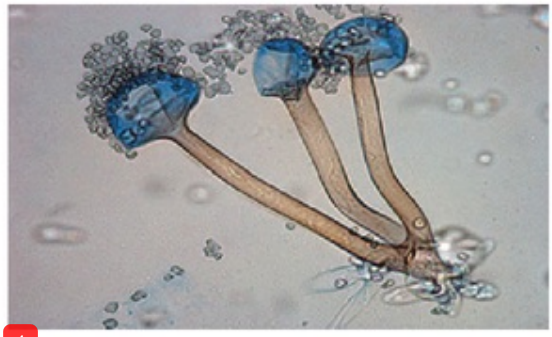

**Fig. 14.5** *Rz. oligosporus* (http://www.mycology.adelaide.edu.au)

### 14.3.3 Microbes Involve in Tempe Manufacture

The microbes in *tempe* are complex, involving mixed culture fermentation by molds, yeasts, lactic acid bacteria, and various other bacteria. The major genus of importance is the mold *Rhizopus* with different species such as *Rz. microsporus*, *Rz. oligosporus*, and *Rz. oryzae* (Nout and Kiers 2005).

*Rz. oligosporus* is a species that can grow between 30 and 42 °C (optimum 25–37 °C), characterized by an inability to ferment sucrose; high proteolytic and lipolytic activity; and ability to release free ammonia after 48–72 h fermentation (Wang and Hesseltine 1965, 1979; Steinkraus 1983), grow on wheat or other cereal substrates without producing noticeable amount of organic acids due to minimal amylase activity (Wang and Hesseltine 1979), inhibit production of aflatoxin, and biosynthesize B vitamins (Murata et al. 1968).

Lactic acid bacteria play a role in the acidification of the soya beans during soaking, thereby preventing the growth of spoilage microorganisms (Ashenafi and Busse 1991; Nout et al. 1987), improving the shelf life of *tempe*. During fermentation, lactic acid bacteria grow up to $10^9$ cfu/g-1 in final *tempe* products.

Samson et al. (1987) reported that the microbial load of 110 commercial *tempe* samples in the Netherlands was more than $10^7$ cfu/g aerobic plate counts, predominated by Enterobacteriaceae and lactic acid bacteria. Sixty-nine percent of the samples contained yeast more than $10^5$ cfu/g. Some samples also contained *Staph. aureus*, *B. cereus*, or *E. coli*. Ashenafi (1994) found high numbers of enterobacteria, enterococci, and staphylococci, whereas Mulyowidarso et al. (1991) found high numbers of *Bacillus* species in *tempe*. The contribution of bacteria and yeasts to the properties of *tempe* is in developing flavor and substrate modification and in the safety of the product (Nout and Rombouts 1990).

## 14.4 Biochemical Changes and Nutritional Value

During fermentation, there are some changes in the chemical composition of the soybeans. The mold, *Rhizopus* spp., produces a variety of carbohydrases, lipases, and proteases, which degrade the macronutrients into substances of lower molecular mass, with a higher water solubility. Also vitamins, phytochemicals, and antioxidative constituents are formed (Astuti et al. 2000; Nout and Kiers 2005).

Soaking, washing, dehulling, and cooking cause considerable loss of solids due to solution into the water (Steinkraus et al. 1960). Fermentation of soybeans by the *tempe* mold also causes an increase in soluble solids from 13.0 % in cooked soybeans to 27.5 % in *tempe* (Steinkraus et al. 1960) and explains the higher digestibility of *tempe* compared to plain cooked soybeans as stated by VanVeen and Schaefer (1950). During fermentation, the pH gradually increases from 5.0 to 7.5 due to ammonia production in the later stages of fermentation (Hand 1966). Fresh, properly fermented *tempe* has been reported to have a pH value of 7.25 (Ilyas et al. 1970). The strains with good amylolytic activity are unsuited for fermentation of *tempe* since they will break down starch to simple sugars which are then used to produce organic acids which will lower the pH and inhibit the growth of the mold.

The main sugars in soybean are sucrose, stachyose, and raffinose, and the last two are oligosaccharides which are considered primarily responsible for flatulence. Soaking and boiling treatment can reduce stachyose, raffinose, and sucrose at the amount of 51 %, 48 %, and 41 % of the original content, respectively (Kasmidjo 1989/1990). Shallenberger et al. (1976) reported further decrease of stachyose and sucrose during the fermentation of *tempe* which might be due to the activity of bacteria, since *Rz. oligosporus* is not able to utilize stachyose, raffinose, and sucrose. Sorenson and Hesseltine (1966) reported that glucose, fructose, galactose, and maltose supported excellent growth of the mold. On the other hand, raffinose was relatively constant.

The amino acid composition of soybeans apparently is not significantly changed by fermentation, but free amino acids and ammonia increased (Wang et al. 1968). Lysine and methionine have been found to decrease during the course of long fermentation. Wang et al. (1968) also found insignificant increase in PER of soybeans after fermentation, which might be attributed to better availability of amino acids liberated from the beans during fermentation and to better digestibility due to increase in soluble solids and nitrogen.

Hesseltine (1965) stated that the total fat (ether extractable) of tempe remained relatively constant throughout fermentation, although about one third of soybean oil was hydrolyzed due to its lipolytic activity into fatty acids by the *tempe* mold into palmitic, stearic, oleic, linoleic, and linolenic acids, with linoleic acid predominant. Linolenic acid is the only fatty acid utilized by the mold, and about 40 % of this fatty acid is used (Hesseltine 1965). The lipid content of *tempe* is lower than that of unfermented soybeans since the lipase enzyme hydrolyses triacylglycerol into free fatty acids around 40–50 % during soybean fermentation (Pawiroharsono 1997). Furthermore, the fatty acids are used as a source of energy for the mold. During *tempe* fermentation the lipid contents decrease about 26 % (Astuti 1994). A study by Graham et al. shows that the mold of *Rz. oligosporus* and *Rz. stolonifer* uses linoleic acid, oleic acid, and palmitic

acid as energy sources, which rapidly decrease during fermentation, and palmitic acid, stearic acid, and linoleic acid by 63.4, 59.25, and 55.78, respectively (Astuti 1994).

Fatty acids in soybean are rich in unsaturated fatty acids (around 80 %), mainly oleic acid, linoleic acid, and linolenic acid. The concentration of oleic acid and linoleic acid increases proportionally with duration of fermentation time, but linolenic acid is decreased, and the optimal concentration is achieved on 24 h of fermentation (Wagenknecht et al. 1961).

A 30.7 % reduction of phytic acid occurred in the *tempe* fermentation (Egounlety and Aworh 2003), while Van der Riet et al. (1987) reported that phytic acid was reduced by about 65 % as a result of the action of phytase enzyme produced by *Rz. oligosporus*. Phytic acid is known as an antinutrient factor which is able to bind divalent minerals, thus lowering the mineral bioavailability. Therefore, the decrease in phytic acid has a beneficial effect on mineral bioavailability (Wang et al. 1980; Astuti 1994).

*Rz. oligosporus* caused almost complete destruction of phytic acid, due to phytase activity of the mold and improved bioavailability of iron (Sudarmadji and Markakis 1977; Fardiaz and Markakis 1981; Sutardi and Buckle 1985). Phytates adversely affect nutritional status by chelating minerals and making them unavailable for use by humans.

## 14.5 Nutritional Value of *Tempe*

*Tempe* contains 157 calories per 100 g, proteins (12.7 %), carbohydrates and fats (4 %), and vitamins $B_1$ (0.17 mg) and $B_{12}$ (2.9 μg); it is low in cholesterol and saturated fat; is high in fiber and most B vitamins including B12; and has good-quality protein (19.5 % ), comparable to protein content of meat products(Shurtleff and Aoyagi 1979; Okada 1989). On a 40 % dry solids basis (Steinkraus 1983), it contains all essential amino acids and is rich in lysine which is lacking in cereal grains, but methionine is limited (Shurtleff and Aoyagi 1979). Wang et al. (1968) found that the nutritive value of *tempe* made from a mixture of soybean and wheat was comparable to that of milk casein. *Tempe* fermentation does not alter amino acid profiles but make them bioavailable. The protein content and nutritive value make *tempe* a good substitute for meat (Steinkraus 1983). During the fermentation process, the levels of anti-nutritional constituents are decreased, and the nutritional quality and digestibility of the fermented product are improved due to the enzymatic activity of the mold (Nout and Kiers 2005). The mold also contributes to the development of a desirable texture, taste, and aroma of the product (Hachmeister and Fung 1993).

### 14.5.1 Antibacterial and Enzymes in *Tempe*

The high digestibility of *tempe* has been observed during World War II when prisoners suffering from dysentery were able to digest *tempe* much better than soya beans (Steinkraus 1996; Tibbott 2004). Pediatric research in Indonesia indicated that in infants, the recovery after acute bacterial diarrhea was faster when *tempe* was consumed as an ingredient of the infant food formula (Karyadi and Lukito 1996, 2000; Soenarto et al. 1997). *Tempe* showed a strong bioactivity in vitro by reducing the adhesion of enterotoxigenic diarrhea-causing *E. coli* to animal and human intestinal cells (Kiers et al. 2002; Roubos-van den Hil et al. 2009), and *tempe* intake was associated with better memory (Hogervorst et al. 2008).

*Tempe* has antibacterial effect against *Lb. bulgaricus*, *Strep. thermophilus*, *Bacillus* sp., and *Listeria* sp., although growth of *Lb. plantarum*, isolated from *tempe*, was not affected. No antibacterial activity of *tempe* against *E. coli* or *Salmonella* was observed (Kiers et al. 2002; Kobayasi et al. 1992; Wang et al. 1969, 1972). *Tempe* was found to possess anti-diarrhea-associated bacteria. On the one hand, *tempe* inhibits the adhesion of ETEC to intestinal cells, which can be of interest in the recovery and prevention of diarrhea in humans. On the other hand, *tempe* has antibacterial against *B. cereus* cells and spores, which can be of interest in food preservation and pathogen control. The anti-adhesion

activity is caused by an interaction between ETEC and *tempe* extracts, which results in a loss of adhesion capability of ETEC to the intestinal cells (Roubos-van den Hil et al. 2009). This bioactivity is found in *tempe* derived from leguminous seeds, but not with *tempe* derived from cereals. The bioactive component(s) are released or formed during fermentation by enzymatic degradation of leguminous matter (Roubos-van den Hil et al. 2010). Fermentation with several other microorganisms also resulted in the formation of bioactive components, such as carbohydrate and arabinose, which is an important monosaccharide constituent supposed to originate from arabinan or arabinogalactan chains of the pectic cell wall polysaccharides of legumes (Roubos-van den Hil et al. 2010). *Tempe* contains enzymes with thrombolytic activity, which can digest thrombotic protein (fibrin) and which will be inactivated by heating above 65 °C (Sumi and Okamoto 2003). Aoki et al. (2003) revealed that tempe contains γ-aminobutyric acid that suppresses the elevation of blood pressure; contains dietary fiber, saponins, isoflavones, and superoxide dismutase which eliminates active oxygen; and has an anticarcinogenic effect.

### 14.5.2 Socioeconomy of *Tempe*

*Tempe* is an indigenous fermented food of Indonesia and the most extensively studied worldwide. For most of Indonesian people, *tempe* is a meat substitute, and the price is affordable for everyone. Throughout Indonesia *tempe* is consumed by people of low as well as high socioeconomic level. The *tempe* makers produce at home using 10–150 kg of soybean daily, and the producers are united in Cooperatives of Producers of *Tempeh* and *Tofu* in Indonesia. Urban population growth has stimulated a rise in the number of *tempe* processors in many cities throughout Indonesia, in response to the demand for relatively inexpensive foods. The significance of *tempe* industry as part of informal sector plays an important role. *Tempe* was formerly considered as an inferior food due to its low costs compared to other protein foods such as meats, fish, and eggs. Over the last four decades, the attitude toward *tempe* has changed, and it is now considered as inexpensive food with high nutritive values (Syarief 1997).

In 1982 a company in Southern Netherlands was making 6000–8000 lb/week of *tempe*, making it the largest *tempe* manufacturing company in the world. In early 1979, there were 13 commercial *tempe* shops in the United States, one in Canada, and four in the Netherlands (Shurtleff and Aoyagi 1979). The total sales of refrigerated *tempe* as meat alternatives in the natural food channel for the year ending August 2011 were at least $51.6 million, and 19.3 % of this was refrigerated *tempe*, while in the mainstream/mass market (including conventional supermarket chains), sales of refrigerated meat alternatives for the year ending August 2011 were at least $65.9 million, and 4.47 % of this was refrigerated *tempe* (Shurtleff and Aoyagi 2011).

In Indonesia, *tempe* is consumed as a protein-rich meat substitute by all economic groups due to its low-cost production, low price, and nutritional value (Karyadi and Lukito 1996). Outside Indonesia, *tempe* gains interest as a major protein source other than meat, especially nutritional and health functionality (Astuti 2000; Nout and Kiers 2005; Steinkraus 1996). Rumah *Tempe* Indonesia (RTI) or Indonesia *Tempe* House was launched on the 6th of June 2012 in Bogor, West Java Province, as a model of Tempe factory for the efficient, hygienic, and eco-friendly issue, implementing good manufacturing practice and good hygiene practice.

## 14.6 *Oncom* and *Gembus*: Microbiology, Nutritive Value, and Potential Health Benefit

Fermentation on solid waste of soybean, peanut residue, or shredded coconut residue also conducted in Indonesia produced *oncom*, *gembus*, and *bongkrek*, respectively. *Oncom* is one of the traditional fermented foods of West Java, a Sundanese ethnic cuisine of Indonesia, involving several molds and closely related to *tempe*.

**Fig. 14.6** *Oncom* Bandung

*Oncom* is made from the by-products of tofu or peanut press cake residue after the oil has been pressed out and cassava tailings when extracting the starch (Fig. 14.6). There are two kinds of *oncom*: *red oncom* and *black oncom*. The solid by-product of tofu or peanut press cakes covered with massive coat of living conidia is called red oncom because of the glistening orange color of the conidia of the microorganisms, and the thicker the conidial layer, the higher the commercial value of the product (Sastraatmadja et al. 2002; Wood 1998).

Since *oncom* production uses by-products to make food, it increases the economic efficiency of food production. Black oncom is made by using *Rz. oligosporus* and other types of *Mucor* (Sastraatmadja et al. 2002; Wood 1998), while *red oncom* is made by involving *Neurospora*, particularly *N. crassa*, *N. intermedia* var. *oncomensis*, and *N. sitophila* (Hoo 1986). It is the only human food produced from *Neurospora*. *N. intermedia* var. *oncomensis* had bright yellow and large macroconidia in contrast to wild *N. intermedia* with pink and small macroconidia (Hoo 1986).

In the production of *oncom*, sanitation and hygiene are important to prevent bacterial or mold contamination such as *A. flavus* (which produces aflatoxin), even though aflatoxin-producing molds (*Aspergillus* spp.) are naturally present on peanut press cake when the peanut has already contaminated with the molds. *N. intermedia* var. *oncomensis* and *Rz. oligosporus* reduce the aflatoxin produced by *A. flavus* (Nout 1989). Soybean is the best substrate for growing *Rz. oligosporus* to produce *tempe*, but *oncom* has not been as thoroughly studied.

Tofu *oncom* is made from soybean residues and peanut *oncom* is based on peanut, well-known traditional fermented foods in West Java. The *oncom* cultures *N. intermedia* var. *oncomensis* had bright yellow and large macroconidia. Generally, red *oncom* is made from solid tofu waste, i.e., the soy residue after its protein has been taken for tofu making, while the black *oncom* is generally made from the peanut dregs mixed with cassava dregs or cassava powder, i.e., tapioca, in order to make a better texture and to make it more tender. Although both the substrate material is a kind of waste, its nutrient is still high enough to be exploited by human. Tofu waste still contains high nutrient values; however, most of its organoleptic properties are less preferred. Fermented *tofu* waste, i.e., red *oncom*, is preferred as food product than the waste without fermentation.

Tofu waste might contain protein similar to *tofu* and soy, although it has undergone many changes because of certain treatments during the manufacturing process of *tofu*, such as heating. The flavor of *oncom* can be described as strong, fruity, almond-like, and somewhat alcoholic, but when fried, it takes mincemeat flavor, while the alcoholic flavor which is present due to sugar degradation will vaporize and disappear. The best *oncom* in Indonesia is *oncom Bandung* (Fig. 14.6); instead of using peanut press cake, raw peanuts are used as the main ingredients.

The high nutrient content of *tofu* and its large amounts provide a significant opportunity to be used as a growth media for enzyme-producing microbes for health. *Oncom* has 187 kcal per 100 g, protein 13%, fat 6%, carbohydrate 22.6%, vitamin $B_1$ 0.09 mg, and vitamin $B_{12}$ 3.1 μg

(Winarno 1989). *In vivo* study revealed that *red oncom* reduces the cholesterol levels of rats, suggesting potential health benefit for humans. The fibrinolytic activities in *oncom* also show potential prevention toward cardiovascular diseases. *Bacillus licheniformis* RO3 with high fibrinolytic activities was isolated from red *oncom* (Afifah et al. 2015).

*Gembus* is also made from solid soybean waste of *tofu* (Kuswanto 2004), fermented by *Rz. oligosporus*, involving *B. pumilus* 2.g which has high proteolytic and fibrinolytic activities (Afifah et al. 2013). *Gembus* is a variety of *tempe*, but whose substrate is different (solid tofu waste and soybean, respectively). Microbial fibrinolytic enzymes from food-grade microorganisms have the potential to be developed as additives for functional foods and as drugs to prevent or cure cardiovascular diseases (Afifah et al. 2014).

Like the soy *tempe*, *gembus tempe* contains several substances such as fiber, polyunsaturated fatty acids, ergosterol, and isoflavonoids, which may have influences on lowering the level of blood lipids (Sumi and Yatagai 2006).

*Gembus tempe* contains 65 calories, protein (3.41 %), carbohydrate (11.94%), fat (0.2%), calcium (143 mg), iron (0.4 mg), and vitamin $B_1$ (0.09 mg) (Sulchan and Nur 2007). Sulchan and Rukmi (2007) reported that *gembus tempe* contains energy (77.70 kcal), protein (4.07 g), lipid (0.23 g), total carbohydrate (14.25 g), fiber (4.69 g), ash (0.84 g), calcium (159.98 mg), phosphorus (59.69 mg), iron (0.48 mg), and water (6 %).

*Gembus tempe*, which is made of the solid soybean waste of tofu (Kuswanto 2004), rich in fiber (4.69 %), and contains a threefold greater level in fiber compared to *tempe* (1.40 g %). The amount of essential fatty acid content in *gembus tempe*, mainly linoleic and linolenic acid, 21.51 % and 1.82 %, respectively (Fatimah 1998).

Sulchan and Rukmi (2007) reported that *gembus tempe* did not contain cysteine, proline, and tryptophan, whereas methionine was found at 11.9 mg/100 g, in extreme contrast with *tempe* containing cysteine (70 mg/100 g) and methionine (168 mg/100 g).

*Tempe bongkrek* is a freshly fermented coconut press cake or shredded coconut residue by *Rz. oligosporus*. Hygienic conditions should be taken into consideration in preventing *P. cocovenenans* contamination and outgrowth of the mold that produces two toxins, toxoflavin and bongkrek acid.

Consumption of *tempe* bongkrek is associated with a food-borne human intoxication and significant numbers of deaths annually. Since 1975, *tempe bongkrek* production has been banned by local authorities for safety reasons.

Garcia et al. (1999) found that 40 % and 50 % coconut fat concentrations in the substrate (shredded coconut residue from coconut milk production) support production of 1.4 mg/g bongkrek acid, while less than 10 % coconut fat supporting growth of the *P. cocovenenans* yields no bongkrek acid. Oleic acid was most stimulatory in production of bongkrek acid (2.62 mg/g dry substrate). Lauric, myristic, and palmitic acids also stimulated production of bongkrek acid but at lower levels.

## 14.7 *Tauco* (*Miso*-Like Product)

*Tauco* is a yellow-colored saline paste, Indonesian style *miso*, made from fermented yellow soybeans and a yellow-colored saline paste with a meat-like flavor and used in Chinese and Indonesian cuisines as flavoring agent (Fig. 14.7). The name comes from its pronunciation in the Hokkien dialect of the Chinese language, and it originates from China. *Tauco* is often used as condiment and flavoring for stir-fried dishes of Indonesian cuisine traditions such as Sundanese and Javanese cuisines. Cianjur town is the center of *tauco* production.

To make *tauco*, the soybeans are soaked in fresh water, the hulls are removed, and the seeds are boiled and spread on bamboo trays to cool. Rice or glutinous rice flour is roasted until golden brown, then mixed with the seeds, and set aside for 3–5 days to ferment between hibiscus (*waroe*) leaves on flat trays, involving mold in the fermentation by *Rz. oligosporus*, *Rz. oryzae*, and *A. oryzae* followed by brine (20 %) fermentation for 20–30 days involving *Lb. delbrueckii* and *Hansenula* sp. After the second phase of fermen-

**Fig. 14.7** Viscous liquid *tauco*, sweet *tauco* (*left*), salty *tauco* (*right*)

tation is completed, the brine is drained; palm sugar (25 %) is added and the mixture is cooked and stored for 24 h or placed directly into bottles. Microorganisms present in *tauco* are *A. oryzae*, *Rz. oligosporus*, *Rz. oryzae*, *Hansenula* sp., *Zygosaccharomyces soyae*, and *Lb. delbrueckii* (Winarno et al. 1973).

When the mass has molded, it is sun-dried for a few days until very hard, and the soybean *koji* for making *tauco* is used. Remove the leaves and put this mass of soybean *koji* into salt water. On the third or fourth day, add some yeast and some cane sugar syrup. Continue the soaking and fermentation in salt water for 3–4 weeks. *Tauco* is available in viscous liquid form (Fig. 14.7) or semisolid form which is obtained by sun-drying the liquid product to a final moisture content of 25 %.

## 14.8 *Kecap* (Soy Sauce)

In the nineteenth century, sinologist Samuel Wells Williams wrote that in China, the best soy sauce is "made by boiling beans soft, adding an equal quantity of wheat or barley, and leaving the mass to ferment; a portion of salt and three times as much water are afterwards put in, and the whole compound left for 2 or 3 months when the liquid is pressed and strained" (Williams 1848).

*Kecap* is an Indonesian soy sauce and usually traditionally made by small-scale producers, with little or no innovation in the process since ancient times. Black soybeans are boiled to undergo spontaneous solid-state fermentation (SSF) before being subjected to brine fermentation. After the brine is filtered, the filtrate is boiled together with caramel and spices, yielding the final product, *kecap* (Roling et al. 1994).

It is a liquid, brown-colored condiment, made by a two-stage batch fermentation which involves the biochemical activities of mold (*Rz. oryzae* or *Rz. oligosporus*), lactic acid bacteria (*Lactobacillus* sp.), and yeast (*S. rouxii*). There are two types of *kecap*, sweetened soy sauce (*kecap manis*) and salty soy sauce (*kecap asin*). According to Codex Alimentarius Commission (FAO/WHO 2004a, b), soy sauce is a clear liquid seasoning obtained by fermentation of soybean and/or by hydrolysis of soybean or other vegetable protein sources to produce soy extract which is further processed into sweet soy sauce or salty soy sauce.

Naturally brewed soy sauce is the product obtained by *A. oryzae* and/or *A. sojae* and/or *Rz. oryzae* and/or *Rz. oligosporus* as main starter(s) and cultured in either soybean or soybean and cereal grains with or without addition of bacteria and/or mold and/or yeast and/or enzyme. Non-brewed soy sauce is the product obtained by hydrolization of soybean and/or other vegetable protein by using acids or enzymes in the brine or salt water, namely, "Hydrolyzed Vegetable Protein," and classified as delicious agents (taste

enhancer agents), not included in the category of soy sauce (FAO/WHO 2004a, b). While mixed soy sauce is the product obtained by brewed soy sauce and hydrolyzed vegetable protein, proportion added by brewed soy sauce is not less than 50 % (FAO/WHO 2004a, b).

*Kecap* is made by spreading cooked soybeans on a bamboo tray and leaving for a period to make molded soybeans (*kecap koji*). The molded soybeans are then mixed with salt solution to carry out the second stage of fermentation under 20 % brine solution for 14–120 days at room temperature (Steinkraus 1995). Then the fermented mash is filtered. To make *kecap manis*, the filtrate is mixed with palm sugar and spices, boiled for 4–5 h and filtered (Steinkraus 1995). *Kecap manis* contains 26–65 % carbohydrate, 0.3 % total nitrogen, and 3–9 % salt (Judoamidjojo 1986).

*Kecap* manufactured in home industry does not usually use any inoculum in *kecap koji* preparation; molds grow on the surface of cooked soybeans as the result of infection from the environment such as the air and the previously used trays (Judoamidjojo 1986; Nikkuni et al. 2002; Steinkraus 1995). Molds isolated from *kecap koji* were mostly of *Aspergillus* sp. (Judoamidjojo 1986; Nikkuni et al. 2002), and aflatoxin producers were found from Indonesian soybean *koji* samples (Nikkuni et al. 2002). According to Sadjono et al. (1992), approximately 47 % of 32 samples of traditionally fermented Indonesian *kecap* tested contained aflatoxin $B_1$ at more than 5 μg/kg. Therefore, the possibility of aflatoxin contamination cannot be ruled out in traditional *koji* making process, and it is thus necessary to use a pure culture starter for food safety concern.

*Kecap* mash prepared according to the traditional method is as follows: black soybeans (40 kg) were soaked in water overnight, boiled for about 3 h, spread on ten bamboo trays (ca. 90 cm in diameter), inoculated with 120 g of the starter culture, and left for 3 days in the koji fermentation room at room temperature by inoculated starter culture. The molded soybeans (*kecap koji*) were sun-dried for 2 days, winnowed to remove the hulls and spores, and placed in a plastic pail, and 70 L of hot water (58 °C) and 30 kg of salt were added to prepare *kecap* mash and allowed to ferment for 2 months at room temperature with exposure to sunlight.

### 14.8.1 Preparation of *Kecap Koji*

*Kecap koji* were prepared without inoculum by the conventional method in home industry, but in the factory, using the starter culture, hence, the *kecap koji* fermentation in the factory is faster than in home industry, 3 and 9 days, respectively. *Koji* culturing is in a mixture of equal amount of boiled soybeans and roasted wheat to form a grain mixture, then *Aspergillus* spores are added (the cultures are called *koji* in Japanese) (Judoamidjojo 1986). After sun-drying, the moisture contents decreased to 7–8 %.

### 14.8.2 *Kecap* Mash Fermentation

*Kecap* mashes were prepared with *kecap koji* and allowed to ferment for 2 months; the pH value of the mash reached 5.5 and contained about 21 % salt. The contents of formol nitrogen and water-soluble nitrogen increased with the fermentation time and showed higher as compared to without the starter culture. The molds involve in brewing soy sauce are *A. oryzae* and *A. sojae* (Fig. 14.8), strains with high proteolytic capacity (Maheshwari et al. 2010). *S. cerevisiae* is also involved, and the yeast will convert some of the sugars to ethanol, and further biochemical changes contribute to flavor development of soy sauce. *Bacillus* sp. may also grow in soy sauce ingredients and generate odors and ammonia, while *Lactobacillus* species will produce lactic acid and lower the pH.

### 14.8.3 Traditional Brine Fermentation

The *Aspergillus* sp. breaks down the grain proteins into free amino acid and protein fragments and starches into simple sugars. This amino-

**Fig. 14.8** Molded soy and wheat by *A. sojae* cultures in traditional fermentation of kecap (Source https://ja.wikipedia.org/wiki/%E3%83%95%E3%82%A1%E3%82%A4%E3%83%AB:Shoyukoji.jpg)

glycosidic reaction gives soy sauce its dark brown color.

### 14.8.4 Brine Fermentation at Industrial Manufacturer

After 43 h SSF followed with brine fermentation for 4 months. About 8000 kg SSF material is mixed with 16,000 l brine. Final salt concentration of the brine is 15 %. Three overlapping phases occurred in brine fermentation: first, amino acid production (based on formol nitrogen production), followed by lactic acid fermentation (based on acetate and lactate production), and lastly yeast fermentation (based on ethanol and glycerol production) (Roling and van Verseveld 1996).

### 14.8.5 Amino Acid Production

Amino acid production started directly after the preparation of brine, and amino acids were produced during the first 3 weeks, and glutamic acid, mainly responsible for flavor, is produced during a longer period, and less than 15 % of the final glutamic acid is formed during the last 3 months and continued to increase slowly after 4 months (Roling and van Verseveld 1996). The activity of glutaminase, which is responsible for conversion of glutamine to glutamic acid, rapidly decreased but did not completely disappear. Even after formol nitrogen production had stopped, protease and leucine aminopeptidase activities were present. Therefore, the exhaustion of digestible proteins was more likely the cause of the termination of amino acid production (Roling and van Verseveld 1996).

### 14.8.6 Fermentation by Lactic Acid Bacteria

Staphylococci and enterobacteriaceae involved in SSF decreased rapidly after addition of salt. Only bacilli was observed after 1 week in brine solution. Numbers of salt-tolerant bacteria were high at the start of fermentation ($10^{7-8}$ cfu/ml), but dropped rapidly within 2 days before increasing again. After 1 week of brine fermentation, only the lactic acid bacterium *T. halophila* was isolated from samples and reached around $10^{8}$ cfu/ml.

The pH of the brine dropped from 5.0–5.1 to 4.4–4.6, and concentrations of lactate and acetate increased up to 164 mM and 69 mM, respectively. Fructose completely disappeared. Lactic acid fermentation took about 2.5–3 weeks, then no further changes in lactate and acetate concentrations occurred, and the number of *T. halophila* declined (Roling and van Verseveld 1996).

### 14.8.7 Yeast Fermentation

Immediately after the preparation of brine, high number of yeast was observed ($10^{4-5}$ cfu/ml). Yeast fermentation in brine fermentation started after 5 days, and in some cases, after 42 days there was no yeast fermentation observed. During yeast fermentation a slight increase in yeast number, ethanol, and glycerol concentrations were observed. *Zygosaccharomyces rouxii* was the dominant yeast species. Glucose concentration dropped during the yeast fermentation, but galactose concentration remained unchanged. In brine fermentation heat-dependent browning reactions (Maillard reactions) took place during the entire

period of fermentation (Roling and van Verseveld 1996).

The filtrate brine undergoes several post-fermentation treatments, such as the addition of caramelized sugar and subsequent boiling for several hours, resulting in a thick, strong brown color and evaporation of volatile compounds such as ethanol.

The organic acids formed during the growth of *T. halophila* have a preserving effect on *kecap*. Amino acids contribute to the flavor of *kecap*, directly as glutamic acid or indirectly via Maillard reactions during boiling of the mixture of brine extract and caramel (Yokotsuka 1986). The amino acid content and lactic acid concentration do not change much after 4 weeks; hence, brine fermentation for 1 month seems to be sufficient for industrial *kecap* production.

Addition of food-grade enzymes in brewing *kecap* represents continuous innovation in production methods and is also conducted by *kecap* manufacturer to speed up the fermentation. The addition of enzymes should be allowed for brewed *kecap*. In traditional *kecap* manufacturing, microorganisms are added for the sole purpose of producing enzymes that hydrolyzed soy proteins for development of the characteristic taste attributes of soy sauce. Whether produced traditionally, or added directly, enzymes carry out the same function. The fully fermented grain slurry is placed into cloth-lined containers and pressed to separate the solids from the liquid *kecap*. The isolated solids are used as fertilizer or fed to animals while the liquid *kecap* is processed further. Finally, the raw *kecap* is pasteurized to eliminate any active yeasts and molds remaining in the soy sauce and then filtered. The *kecap* can be aged or directly bottled and sold (Fig. 14.9).

In traditional practice, the liquid is extracted, clarified, and filtered before introduction of


**Fig. 14.9** *Kecap manis* (sweet soy sauce)

desired taste and flavor by addition of brown sugar, spices, and certain additives (enhancer, preservatives and or coloring, and molasses); finally it is pasteurized and packaged. Sweet soy sauce in Indonesia is mostly produced by medium-large enterprise (approx. 60 %) while the remaining 40 % produced by small-medium enterprise (FAO/WHO 2004b).

Industrial manufacturers use defatted yellow soybean flakes and wheat instead of black soybeans only. SSF is well controlled and inoculated; however, brine fermentation is spontaneous and subjected to tropical weather conditions for 4 months (Wilfred et al. 1996). During brine fermentation in traditional *kecap* manufacture, amino acid production and growth of the lactic acid bacterium *Tetragenococcus halophila* (until recently known as *Pediococcus halophilus*) take place. Few or no obvious yeast fermentation is observed (Roling et al. 1994).

### 14.8.8 Socioeconomic Value

In Indonesia, during 2003, total production of sweet soy sauce and salty soy sauce was approximately 80.000 tons (90 %) and 31.200 tons (10 %), respectively, and the potential growth is about 3.6 % per year. As condiment, consumption of sweet soy sauce is about 0.9 l/capita/year. Besides being part of daily Indonesian cuisine, *kecap* or sweet soy sauce also served as Indonesian typical sauces for instant noodle and other products.

### 14.8.9 Quality Criteria of Soy Sauce

The total nitrogen should be not less than 0.4 % w/w in salty soy sauce and not less than 0.15 % in sweet soy sauce, the soluble solid contents, exclusive of added salt not less than 6 % (w/v), and the sugar content for sweet soy sauce is not less than 30 %, and salt content for salty soy sauce is not less than 10 % (FAO/WHO 2004a).

### 14.8.10 Food Safety Concern on Carcinogens

Soy sauce may contain ethyl carbamate, a Group 2A carcinogen (Matsudo et al. 1993). In 2001, the UK Food Standards Agency found that 22 % of tested samples contained a chemical carcinogen named 3-MCPD (3-monochloropropane-1,2-diol) at levels considerably higher than those deemed safe by the EU (0.02 mg/kg) from various soy sauces manufactured in mainland China, Taiwan, Hong Kong, and Thailand, made from hydrolyzed soy protein, rather than being naturally fermented (Hamlet et al. 2002; Crews et al. 2003). About two thirds of these samples also contained a second carcinogenic chemical named 1,3-DCP (1,3-dichloropropan-2-ol) which should not be present at any levels in food. Both chemicals have cancer potential, and the agency recommended to withdraw from shelves and avoided 3-MCPD and 1,3-DCP, chloropropanol carcinogens. The same carcinogens were found in soy sauces manufactured in Vietnam, causing a food scare in 2007. Continuous lifetime exposure to high levels of 3-MCPD could pose a health risk, and Health Canada has established 1.0 part per million (ppm) as a guideline for importers of the sauces and considered to be a very safe level (Fu et al. 2007).

## 14.9 Fermented Fruits and Vegetable Products

### 14.9.1 *Acar* Pickles

*Acar* is a type of vegetable pickles (Fig. 14.10) made in Indonesia, Malaysia, and Singapore, usually prepared in bulk as it easily is stored in a well-sealed glass jar in refrigerator for a week and served as the condiment for any meals. It is a localized version of the Mughlai Achaar. It is known as *atjar* in Dutch cuisine, derived from Indonesian *acar*. In Indonesia, *acar* is commonly made from small chunks of cucumber, carrot,

**Fig. 14.10** Mixture of cucumber and carrot *acar*

shallot, young bamboo shoot, eggplant, chili, and occasionally pineapple and marinated in a sweet and sour solution of sugar and vinegar.

*Acar* is a very popular accompaniment in many of Indonesian dishes, such as *nasi goreng* (fried rice), friend noodle, *sate*, and almost all varieties of *soto*. It is very easy to prepare at home; the key to a successful *acar* is to use the freshest ingredients possible. Just like common pickles, the sour taste of vegetable acar may freshen up the meal, especially the fishy dish such as grilled fish or the rich and oily dish such as *mutton satay* to neutralize the fatty taste.

Various microorganisms are usually associated with fresh fruits and vegetables as normal flora, transit flora, spoilage, and pathogenic organisms. Cucumber comes in contact with soil insects and animals during its growth and harvest from the field, and therefore, the microbial flora will include soil microorganisms and those from contaminated irrigation water; direct contamination by wild animals, birds, and insects; and transportation with contaminated containers (Reina et al. 2002; Heaton and Jones 2007; Williamson et al. 2003).

Cucumber (*Cucumis sativus*) is one of the primary vegetables often fermented to obtain pickles involving a mixed microbial fermentation in which desirable and undesirable bacteria and fungi interact and compete during the initial stages of the fermentation. The harvested cucumbers were naturally fermented in 10% (w/v) NaCl solution for 30 days with and without $CaCl_2$ and/or polygalacturonase (PG). $CaCl_2$ and PG treatments did not interfere with fermentation. Separately, $CaCl_2$ enhanced firmness of pickles while PG was effective in causing excessive softening. When $CaCl_2$ was present in PG-containing solutions, softening by PG was inhibited (Lennox and Efiuvwere 2013; Buescher et al. 1979).

#### 14.9.1.1 Microbial Changes During Acar Fermentation

The common microorganisms usually isolated from cucumber are enteropathogenic bacteria, lactic acid bacteria (LAB), *Pseudomonas* spp., *E. carotovora*, and some fungi, and the heterotrophic plate counts in the produce range between $4.0\times10^2$ and $5.7\times10^2$ cfu/g-1 (Nahaisi et al. 2005).

The changes in microflora of the fermenting cucumber in brine solution were reported by Lennox and Efiuvwere (2013), with the initial counts of $4.2\times10^6$ cfu/ml, $1.4\times10^3$ cfu/ml, $5.0\times10^6$ cfu/ml, and $1.2\times10^3$ cfu/ml for lactic acid bacteria (LAB), fungi, *E. coli*, and *Salmonella–Shigella*, respectively, in the fermenting brine solution. There was sharp increase in counts of LAB and indicator organisms in the brine on the third day, and thereafter they started to decline but the indicators were finally inhibited by the 12th day. *Salmonella–Shigella* showed very slight increase on the third day but were finally inhibited by the 12th day. The fungi counts fluctuated and reached their peak on the sixth day, but were finally inhibited also by the 15th day. LAB persisted to the end of the fermentation with the total count of $2.1\times10$ cfu/ml. There was no growth of *Salmonella–Shigella* within the fruit during fermentation. On the third day of fermentation, only LAB and indicator organisms grew with counts of $5\times10^3$ cfu/g and $3\times10^3$ cfu/g, respectively. The indicator organisms were inhibited within the cucumber by the sixth day. The changes in the microbial flora in the brine and cucumber could have been due to the competitive nature of the microorganisms in any environment and proved to be effective in eliminating pathogenic organisms and other contaminants and also preserved the cucumber.

There is usually succession of LAB during fermentation of pickled cucumber. The epiphytic LAB which occur naturally on the surface of the cucumber initiate fermentation and effectively

control the microbial ecology of the fermentation by consuming the glucose and fructose present, producing lactic acid, and lowering the brine pH which favors *Lb. plantarum*, a homofermentative, acid-tolerant LAB which takes over the fermentation and does not result in production of carbon dioxide from sugars (Lennox and Efiuvwere 2013). The lactic acid they produce is effective in inhibiting the growth of other bacteria that may decompose or spoil the cucumber due to metabolic products such as lactic acid like bacteriocins, peroxides, and peptides that can inhibit other bacteria (DeVuyst and Vandamme 1994; Sapers and Annous 2004).

### 14.9.2 *Sayur Asin*

*Sayur asin* (Fig. 14.11) is an ethnic, fermented mustard cabbage leaf (*Brassica juncea* var. *rugosa*) product from Indonesia (Puspito and Fleet 1985). Mustard cabbage leaves are sorted, washed, withered, wilted, and rubbed or squeezed with 2.5–5 % salt. Liquid from boiled rice (*air tajin*) is added to provide fermentable carbohydrate to assure that sufficient acid is produced during fermentation. Fermentation is initiated by *Leuc. mesenteroides*, *Lb. confusus*, and *Lb. curvatus* and later dominated by *Lb. plantarum* and *P. pentosaceus*. Starch degrading species of *Bacillus*, *Staphylococcus*, and *Corynebacterium* exhibited limited growth during the first day of fermentation. The yeasts, *C. sake* and *C. guilliermondii*, also contributed to the fermentation. The pH falls from 6.5 to 4.2 in 8 days of fermentation (Puspito and Fleet 1985). Lactic acid, acetic acid, succinic acid, ethanol, and glycerol are produced during 2–14 days of fermentation. Hydrolysis of starch and maltose resulted in glucose, which is utilized by microbes for their growth during fermentation.

Manufacture of fermented mustard cabbage leaves was made by addition of the salt to vegetables, allowing the growth of certain fermentable microorganisms, resulting in sensory changes bearing acidic and unique characteristics to the *sayur asin* (Chiou 2004). Epiphytic lactic acid bacteria (LAB) of which initially amounted to slightly between 10 and 1000 cfu/g plant (0.001–1 % of the total population of microorganisms) became dominant within the microorganism population in the fermented mustard cabbage as a result of the anaerobic condition on the vegetables (Daeschel et al. 1987; Azcarate and Todd 2010). After 2 days of fermentation, the lactic acid was produced at 0.8–1.5 % in 2.5 % brine and the pH reached 3.4 (Sulistiani et al. 2014). A combination of acidic conditions and salt concentrations suppressed the growth of undesirable microorganisms, hence, preserving the vegetables.

Pederson (1971) reported that 2.25–2.5 % salt allowed exclusive growth of lactic acid bacteria, suppressed the growth of spoilage bacteria, and inhibited pectinolytic and proteolytic enzymes that can cause softening and putrefaction (Swain et al. 2014); the osmosis process will draw out the water and nutrients from vegetables as growth

**Fig. 14.11** *Sayur asin* on sale in the traditional market

medium, facilitating metabolism of sugar into lactic acid during fermentation. Low salt concentration such as less than 2.25 % will support the growth of proteolytic bacteria, while adding more than 10 % salt will enable the growth of halophilic bacteria and cause fermentation failure. In general the higher the salt concentration, the slower fermentation. For a short time fermentation preferably 2.5–10 % brine solution is used (Swain et al. 2014).

Sulistiani et al. (2014) reported identification of 246 lactic acid bacteria isolates from *sayur asin* based on 16S rDNA sequence data. The bacteria belong to 11 species, viz., *Lb. farciminis* (15 isolates), *Lb. fermentum* (83 isolates), *Lb. namurensis* (18 isolates), *Lb. plantarum* (107 isolates), *Lb. helveticus* (1 isolate), *Lb. brevis* (1 isolate), *Lb. versmoldensis* (3 isolates), *Lb. casei* (12 isolates), *Lb. rhamnosus* (2 isolates), *Lb. fabifermentans* (3 isolates), and *Lb. satsumensis* (1 isolate), and revealed that *Lb. plantarum* and *Lb. fermentum* are common LAB used in *sayur asin* production from Central Java, Indonesia, and have also been reported to be found in different fermented foods (Ludwig et al. 2009). These species have been characterized into different fermentation types: obligately homofermentative, facultatively heterofermentative, and obligately heterofermentative (Felis and Franco 2007), and they have been reported to be not pathogenic to human or animal (Azcarate and Todd 2010). Therefore, the sayur asin is safe for human consumption.

Several species which were not found by Puspito and Fleet (1985) have been determined by molecular identification. These include *Lb. namurensis*, *Lb. versmoldensis*, *Lb. rhamnosus* (previously known as *Lb. casei* subsp. *rhamnosus*), *Lb. fabifermentans*, and *Lb. satsumensis*. The result showed that each sample consisted of various species, predominated by *Lb. plantarum* and *Lb. fermentum*, with *Lb. fermentum* and *Lb. plantarum* being more acid tolerant and often dominating the fermentation processes of vegetables and cereals (Sulistiani et al. 2014). In another study, Swain et al. (2014) reported that *Leu. mesenteroides*, *Lb. confusus*, *Lb. curvatus*, *P. pentosaceus*, and *Lb. plantarum* have been isolated from *sayur asin*. The spontaneous fermentation of *sayur asin* involved diverse variation of lactic acid bacteria.

According to Chao et al. (2009), the variety of LAB population in the fermented mustard was influenced by different fermentation process and treatments, especially the salt concentration. During the process, the squeezed and salt treatment diffused water and nutrient out of the vegetable tissues by high osmotic pressure (Chiou 2004). In addition, the varying conditions of anaerobiosis, moisture levels, and temperature resulted in changes in the population balance and selected for spontaneous fermentation by lactic acid bacteria (Azcarate and Todd 2010). The growth of the lactic acid bacteria is also influenced by nutrient movement from plant material into the surrounding liquid (Daeschel et al. 1984).

### 14.9.3 *Brem*

*Brem* is traditional fermented food or fermented beverage, a non-distilled ethnic alcoholic drink from Indonesia prepared from glutinous rice. It is a dried, starchy, sweet–sour rice extract and is eaten as a snack. There are two types of *brem*: *brem cake* (solid), which is yellowish-white, sweet–sour snack usually eaten in Madiun, where it is prepared in blocks of 0.5×5 to 7 cm (Fig. 14.12), and in Wonogiri (Fig. 14.13), where it is sweet, very soluble, white, and thin circular blocks of 5 cm diameter, and *brem beverage* (liquid), which is made of rice wine from Bali and Nusa Tenggara, but mostly known from Bali (Basuki 1977).

*Brem cake* from Madiun (Fig. 14.12) and Wonogiri (Fig. 14.13) is believed by Indonesian


**Fig. 14.12** *Brem* Madiun

**Fig. 14.13** *Brem* Wonogiri

consumer to be important for stimulating the blood system. It is also reported to prevent dermatitis, probably due to the presence of significant amounts of B vitamins produced by the microorganisms. This product is consumed as a snack and is not part of the daily family diet.

All three types of *brem* are made from the liquid portions of *tapé ketan* (fermented glutinous rice). The glutinous rice is steamed and spread on the trays lined by banana leaves to cool, then 0.2 % powdered *ragi* (inoculum), the same ragi for *tape singkong* fermentation is added to the cooled rice and mixed thoroughly, incubated at room temperature (30 °C) for 3 days aerobically, the juicy rice called tape is pressed out and transferred to the fermenting jars, fermented anaerobically at room temperature for 8–10 weeks. After fermentation, the juice is siphoned carefully into sterilized bottles and stored in a cool room for aging around 8–12 months (Aryanta 1980).

During *brem* production, the filtrate of *tapé ketan* is boiled down, poured onto a table, covered with banana leaves, and left to cool at ambient temperature over 8–12 h (*brem* Madiun) or sun-dried for 1 day to produce *brem* Wonogiri (Campbell-Platt 1987).

*Brem beverage* is a traditional rice wine of Bali island. The process of *brem* making involves a solid-state fermentation of steamed glutinous rice by a traditional inoculum (*ragi*), extraction of the liquor, and further liquid-state fermentation without additional inoculation. The quality of this product is inconsistent due to the inconsistency of the microorganisms in ragi as a consequence of spontaneous fermentation. In the Philippines, rice wine is called tapuy, while in Japan it is called sake (Aryanta 2000).

The liquid portion of *tapé ketan* is aged for 7 months, during which solids precipitate, leaving a clarified brem, known as *brem* Bali, and is decanted and bottled (Basuki 1977). Alcohol content of *brem* Bali (Fig. 14.14) is 6.1 % (Winarno 1986). *Brem* with improved *ragi* is produced which has more desirable flavor than conventionally made *brem* (Saono et al. 1984).

*Brem beverage* consumed and holds important use in temple ceremonies of Hinduism called Tetabuhan, an offering beverage for *Buto Kala* (lit. Kala the Giant) in order to evoke harmony.

*Brem* Bali *beverage* can be either white or red depending on the proportions of white and black glutinous rice used in production; it is very sweet to semisweet, yet acidic, and contains alcohol with varying degree, usually from 5 % to 14 %.

Liquid *brem* is made from fermented mash of black/white glutinous rice using a dry starter

**Fig. 14.14** *Brem* Bali beverage

called *ragi tape*. Glutinous rice is soaked and drained, steamed for 1 h, and then cooled down. The cooled glutinous rice is then inoculated with *ragi tape* and amylolysis begins. A honey-like rice syrup settles in the bottom of the malting vessel. Following 3 days of conversion from the starch to sugar, yeast culture is added and alcoholic fermentation begins. Alcoholic fermentation typically goes on for 2 weeks.

#### 14.9.3.1 Microbiological and Biochemical Changes

Aryanta (1980), Lotong (1985), and Uchimura (1998) reported the microbes in *ragi*. Aryanta (1980) reported that during the first 3 days of fermentation, the population of molds in brem (*ragi* NKL as inoculum) was 3.5 × 105 cfu/ml and decreased to $5.9 \times 10^2$ cfu/ml; the yeast was at $5.5 \times 10^4$ cfu/ml and increased to $4.9 \times 10^6$ cfu/ml, and after 2 weeks of fermentation, it then decreased until the sixth week, and no mold was found. No yeast was isolated at the eighth week of fermentation. On the third day of fermentation, bacterial count was 6.5 × 10 cfu/ml which increased until the second week ($8.0 \times 10^6$ cfu/ml) and then decreased until sixth week. On the eighth week of fermentation, no bacteria were isolated (Aryanta 1980).

The sugar content of *brem* decreased during the fermentation, due to the decomposition of simple sugars into ethanol and carbon dioxide by the yeast's enzyme activity (Pederson 1971) through Embden–Meyerhof–Parnas (Aurand and Woods 1973).

During fermentation of *brem*, the pH decreased, which might be due to more production of some organic acids at longer fermentation time. After 10 weeks, the pH reach 4.0 and contained 3 % reducing sugar, 6 % ethanol, and 0.6 % total acidity (as acetic acid), due to the oxidation of ethanol to acetic acid by the activity of *Acetobacter aceti*.

### 14.9.4 *Tuak* (Palm Wine)

*Tuak* (palm wine) is one of the indigenous alcoholic beverages most widely known in North Sumatra region of Indonesia. The *Arecaceae* such as palm sap of aren (*Arenga pinnata*) and nipa (*Nypa fruticans*) called *nira*, a sweet juice with pH between 5.5–6.5 % and 80–90 % moisture content, is fermented spontaneously through the application of one or more several kinds of woodbark or root, called raru (*Xylocarpus* woodbark or a variety of forest mangosteen), into the sap water of sugar palm (*Arenga pinnata*) with the involvement of natural yeasts for 2–3 days.

The sweet taste of the palm sap is due to the presence of sugars (sucrose, glucose, fructose, and maltose). The sugar content is 12.30–17.40 %, and the reducing sugar is 0.5–1 %. In addition to sugar, the juice contains other ingredients such as protein, fat, water, starch, and ash as well as organic acids (citric, malic, succinic, lactic, fumaric) that play a role in the formation of specific brown sugar flavor (Judoamidjojo 1985). Hence, palm sap is a good medium for the growth of microorganisms such as bacteria, fungi, and yeast and needs to be preserved as soon as possible. The presence of microorganisms may spoil the palm sap which is characterized by the formation of mucus to become turbid, murky, green, white, and frothy sour taste.

In North Sumatra, *Tuak* is produced by spontaneous fermentation of the palm sap (*Arenga pinnata*) in the presence of raru wood or several kinds of woodskin or roots (like nirih – Xylocarpus woodskin or a kind of forest mangosteen) for overnight incubation. Native people in Gorontalo, North Sulawesi, called *tuak* as "bohito" in their native language (Latief and Latief 2014).

Hermansyah et al. (2015) isolated and identified culture independent method for *Candida tropicalis* from North Sumatra's *tuak* among other yeasts. The *C. tropicalis* isolate is able to utilize glucose for more rapid and higher production of ethanol at high temperature of 42 °C as compared with *S. cerevisiae*. However, the optimum temperature of *C. tropicalis* isolates is 30 °C as displayed by its ability to produce 6.55 % (v/v) and 4.58 % ethanol from 100 g/l glucose fermentation at 30 °C and 42 °C, respectively. Rahayu dan Kuswanto (1988) revealed that the alcohol content in *tuak* was 3–10 %, which depends on fermentation medium (*aren* or *nipa*) and fermentation time, and the most important thing is the natural indigenous microbes involved during fermentation. Hartanto (1997) revealed that alcohol content of tuak is quite similar to wine (6–12 %).

#### 14.9.4.1 Social Aspect

*Tuak* contains alcohol; hence, Moslem community does not drink *tuak*. Non-Moslem communities in some areas in Indonesia consume *tuak* and also used it as traditional remedy and in some ritual or traditional ceremony in Sumatra and Flores islands (Ikegami 1997; Ola 2009). The ethnic tribe Batak Toba believe that *tuak* is good for new mothers after giving birth to augment their breast milk production and to remove the impurities through sweat. In North Sumatra, there is traditional ceremony to respect old generation, namely, *manuan ompu-ompu* dan *manulangi* (Ikegami 1997). Likewise, traditional ceremony called *Lewak Tapo* in Lamaholot ethnic group of Adonara island, East Flores. Latief and Latief (2014) reported that in Momala village, Gorontalo, North Sulawesi, a community called *pakua lo bohito* consumes *tuak* for making social interaction, as Japanese drink sake.

**Fig. 14.15** Fermented durian, *tempoyak*

### 14.9.5 *Tempoyak*

*Tempoyak* is a traditional condiment (Fig. 14.15) made from the flesh or raw pulp (aril) of the durian fruit (*Durio zibethinus*), a kind of tropical fruit, naturally fermented at room temperature in a tightly closed container and normally prepared from excess, poor-quality or overripe fruits (Ganjar 2000). This product is popular among people living in Riau Province, Sumatra, Indonesia, as well as in Malaysia. Interestingly, even though the fresh durian pulp is fermented without any heat application, there is no record of food-borne illness caused by the consumption of tempoyak. *Tempoyak* has a long history of safe consumption, in Riau, Sumatra.

The fermentation process may involve salt or without salt, and usually low amount of salt, 1.3 %, is added to support the growth of desired lactic acid bacteria besides yeast as saccharolytic microbes. *Tempoyak* is manufactured by mixing durian pulp with salt and allowing it to ferment for 3–7 days, producing a distinctive durian smell and creamy yellow color with sour and salty taste (the sour taste dominates). *Tempoyak* is consumed with rice or added to cooking dishes as condiment (Ganjar 2000).

The initial pH of *tempoyak* is in the range of 6.62–6.83, and after 2 days of fermentation, the

pH is in the range of 3.96–4.08 (Leisner et al. 2001; Merican 1977; Amin et al. 2004). The total acidity of *tempoyak* is around 3.6 % as acetic acid and the final pH value is 3.8–4.6 (Steinkrauss et al. 1996; Merican 1977). Suan (1996) reported that *tempoyak* has 2.0 % ash, 67 % moisture, 4.5 % total sugar, 2.5 % crude fiber, and 1.4 % fat.

### 14.9.6 Lactic Acid Bacteria Involvement During Tempoyak Fermentation

Several LAB found to be involved in the fermentation of *tempoyak* are *Lb. plantarum*, *Leu. mesenteroides* subsp. *mesenteroides*, *Streptococcus faecalis* (Ohhira et al. 1990), *Leu. mesenteroides*, *Lb. brevis*, *Lb. mali*, *Lb. fermentum* (Leisner et al. 2001), and *Lb. durianis* (Leisner et al. 2002). Wirawati (2002) isolated *Lb. plantarum*, *Lb. coryniformis*, and *Lb. casei* from tempoyak. Yuliana and Dizon (2011) found *Lb. plantarum*, *Lactobacillus* sp., *W. paramesenteroides*, and *P. acidilactici* in *tempoyak*.

In another study, Widowati et al. (2013) reported the involvement of *Oenococcus*, *Leuconostoc*, *Enterococcus*, *Lactococcus*, *Pediococcus*, and *Lactobacillus*. *Leuconostoc* sp. in the presence of 2 % and 4 % salt, respectively, at 20 ± 2 °C during early stages of 4 weeks fermentation, whereas heterofermentative *Lactobacillus* sp. and homofermentative *Lactobacillus* sp. dominated the bacterial population in the middle stage. At the end of fermentation, homofermentative *Lactobacillus* sp. and *Pediococcus* sp. were found during tempoyak fermentation.

While Pato and Surono (2013) isolated *Ent. gallinarum* and *Ent. faecalis*, *out of* 12 isolates classified as genus *Lactobacillus* sp. isolates and the other 32 *Enterococcus* sp. isolates from tempoyak in Riau, . Almost all LAB isolated from *tempoyak* were relatively resistant to acid as indicated by the reduction in the number of colonies between 0.76 and 2.82 log cycles at pH 3.0 after 2 h incubation. These lactic acid bacteria play an essential role in preserving raw food materials, i.e., durian, and contribute to the nutritional, organoleptic, and health properties of *tempoyak*.

Pato and Surono (2013) identified two potential probiotic strains isolated from tempoyak, *Enterococcus* sp. UP-9 and *Enterococcus* sp. UP-11, by PCR the 16S rRNA gene sequences using specific primers and sequencing of amplified region by using an automated sequencer, and showed 97 % homology to *Ent. gallinarum* and *Ent. faecalis*, and named *Enterococcus gallinarum* UP-9 and *Enterococcus faecalis* sp. UP-11, respectively. This finding is contradictory to the previous report by Leisner et al. (2000) where Lactobacillus was the dominant lactic acid bacteria in tempoyak due to its natural fermentation. *Enterococcus* sp. and *Lactobacillus* sp. were the predominant genus in tempoyak and relatively resistant to acid as the isolates were originated from tempoyak with pH 3.69. *Ent. gallinarum* UP-9 and *Ent. faecalis* UP-11 showed potential probiotic properties and were able to reduce cholesterol level by different mechanisms, namely, deconjugating taurocholic acid and cholesterol binding (Pato and Surono 2013).

Amin et al. (2004) reported that addition of 1 % salt in *tempoyak* fermentation for 10 days showed the highest lactic acid bacteria viable counts, and at 8–10 days fermentation, the viable lactic acid bacterial counts were comparable between 1 % and 2 % salt addition. The higher the salt concentration, the lower the lactic acid bacteria viable counts; however, addition of 2 % salt produced the most preferred *tempoyak* by sensory evaluation.

The involvement of lactic acid bacteria in *tempoyak* fermentation might be due to the total sugar content in durian fruit, 15–20 % (Ketsa and Daengkanit 1998), and 17 % saccharose which may favor the growth of lactic acid bacteria and yeast (Leisner 2001).

### 14.9.7 *Mandai*

*Mandai* is a fermented product made from *cempedak* (*Artocarpus champeden*) or jackfruit (*Artocarpus heterophyllus*) inner peel in brine solution, a traditional fermented food of native

people in the province of Central, South, and East Kalimantan. *Dami*, inner part of peel, is the nonedible part of the fruit. The *dami* is cleaned and soaked in a 5–15% brine solution for 2 weeks. Fermented *mandai* is seasoned and consumed as a side dish of rice. It tastes good and is savory and its texture resembles that of meat, making these foods popular. *Mandai* fermentation process is part of an effort to preserve and to utilize the waste from jackfruit consumption. *Mandai* generally can be kept for 1 year or more. Rahayu (2003) found nine isolates of lactic acid bacteria from *mandai* cempedak and identified by molecular detection as *Lactobacillus plantarum* and, *P. pentosaceus*. Emmawati (2014) reported the involvement of *Lb. plantarum* in *mandai* fermentation.

#### 14.9.7.1 Dynamic Changes of Lactic Acid Bacteria and Biochemical Changes During Mandai Fermentation

The total viable count of lactic acid bacteria was reported to increase during *mandai* fermentation in 10% and 15% brine solution, but not in of 5% brine solution. The initial viable count of lactic acid bacteria was approximately 6–7 log cfu/ml and the final viable count was in the range of 7.0–7.7 cfu/ml (Emmawati et al. 2015; Nur 2009) until day 14 of fermentation. Salt concentration significantly influences the amount of lactic acid bacteria. The higher the concentration of salt added during *mandai* fermentation, the higher the viability of lactic acid bacteria counts. Salt in the fermentation of *mandai* added environmental selection factors.

Effect of salinity on the growth of lactic acid bacteria during fermentation was also reported by Ji et al. (2007) in cabbage fermentation. Higher salt concentration will decrease the total viable count of lactic acid bacteria. Eight to 12% brine solution will inhibit the growth of lactic acid bacteria at the beginning of fermentation and then increase at the end of fermentation. Salt affects microbial growth by reducing the availability of water in the cell. The presence of salt also lowers the reduction potential of limiting the growth of aerobic microorganisms and otherwise supports the growth of microorganisms that are microaerophilic and anaerobic (Emmawati 2014). *Mandai* fermentation in 10% and 15% brine solution facilitates the growth of lactic acid bacteria, as shown by higher viable counts of lactic acid bacteria. On the other hand, 5% brine solution may facilitate other microbes to grow, and the competition for the nutrients may suppress the growth of lactic acid bacteria.

Biochemical aspects such as reducing sugar and N-total decreased to 0.240% at day 14 and 0.159% at 21 day, respectively. Substrates for salinity increased in the third week to 4.941% and relatively stable. The pH value of the substrate is in the range of 3.71–6.02 (Emmawati 2014).

The dynamic changes of microbes during *mandai* fermentation was reported by Emmawati (2014) that on days 4–8 of fermentation, cocci isolates predominate the microbe population. On day 8, the viable counts of lactic acid bacteria were decreased in *mandai* fermentation. The lactic acid bacteria isolated from day 8 at 15% brine solution of fermentation reveal that the isolates are halotolerant or halophilic cocci. However, at day 12, the cocci isolates were not found, probably due to acidic environment as a result of more metabolites produced, including organic acids, thus lowering the pH and making the condition to become acidic with the final pH in the range of 4.16–4.8.

### 14.9.8 Low Salt Concentration on Microbial and Biochemical Changes in Mandai Fermentation

In general, *mandai* is made in high salt concentration. Nur (2009) reported the fermentation of *mandai* in 10% (w/v) brine solution for 14 days. Microbial succession occurred during fermentation. Yeast cells grew dominantly ($2.8 \times 10^9$ cfu/g) on day 5, but bacteria were dominant at day 14 ($1.1 \times 10^7$ cfu/g). The highest decrease of reducing sugar and N-total contents were 0.240% at day 14 and 0.159% at day 5,

respectively. The pH value was varied within the range of 3.71–6.12 for the whole period of fermentation.

Biochemical parameters such as reduction sugar, N-total, pH, and salinity of substrates were changed. Reducing sugar content decreased to 0.240 % on day 14 and the levels of N-total also declined in day 5 to 0.159 %. The pH value of the substrate was in the range of 3.71–6.02 (Nur 2009).

The addition of salt to organic substrates leads to a series of spontaneous fermentation and microbial selection that leads to a succession of microbes. Salt in high concentrations can inhibit the growth of spoilage and pathogenic microbes due to the decrease in the value of water activity ($a_w$) and ionized salt into ions Cl toxic. Treatment with high salt on the one hand affects aroma preservation and formation and on the other hand poses a concern for the health of the consumer, especially hypertension.

Rahayu (2003) and Lindayani and Hartayanie (2013) isolated *Lb. pentosus* from *mandai* of Semarang city, which can grow at 10 °C, 45 °C, and 50 °C and at pH 4.4 in 6.5 % brine solution. *Lb. pentosus* is heterofermentative lactic acid bacteria that cannot grow at pH 9.6 and in 18 % brine solution.

## 14.10 Fermented Milk Products

### 14.10.1 *Dadih*

*Dadih* (*dadiah*, in native language) is an Indonesian traditional fermented milk made out of buffalo or cow's milk produced and consumed by the West Sumatran *Minangkabau* ethnic group of Indonesia (Fig. 14.16). It is one of the very popular dairy products in Bukittinggi, Padang Panjang, Solok, Lima Puluh Kota, and Tanah Datar (Surono and Hosono 1996a).

It is a significant dairy product in the diet resembling yogurt and is similar to dahi of India with a distinctive thicker consistency, smooth texture, and pleasant flavor due to its higher total solid content, higher fat content, and casein content as compared to cow's milk. *Dadih* provided

**Fig. 14.16** *Dadih* product

safety, portability, and novelty to milk nutrients for the indigenous people in West Sumatra.

The higher protein content in buffalo milk results in custard-like consistency at the end of fermentation. In addition, higher fat content enriches the flavor developed in the *dadih* products. A good-quality *dadih* is firm with uniform consistency and has a creamy-white color, pleasant aroma, and acidic taste with smooth and glossy surface; its cut surface is trim and free from cracks and air bubbles.

*Dadih* and *dahi* are Indonesian and Indian yogurts, respectively, which seem to share the same root word. The body and texture of yogurt depend largely on the composition of milk employed in its manufacture, whereas the manufacture of *dadih* and *dahi* is simpler than Western-type yogurt, without any starter cultures involved (Surono and Hosono 2011). *Dadih* is served at weddings and during inauguration of an honorable title "Datuk" in West Sumatra during the ethnic tradition or "adat" ceremony. Generally, *dadih* is consumed during breakfast with rice after adding sliced shallot and chili (*sambal*), or it is mixed with palm sugar and coconut milk, being served as a topping of steamed traditional glutinous rice flakes, a corn flake-like product, called *ampiang dadih*.

#### 14.10.1.1 Manufacturing of *Dadih*

The manufacturing method of *dadih* is quite similar to the *dahi* of India, except for the heat treatment of raw milk and the starter cultures being incorporated. In *dahi* making, the raw cow or buffalo milk, or a combination of both, is pasteurized and then fermented using leftover *dahi* from the previous lot as starter cultures (Indian Standard Institution 1980). In Indonesia, *dadih* is a homemade product by the traditional way, involving the milk of water buffaloes without any heat application to buffalo milk while manufacturing. The milk is neither boiled nor inoculated with any starter culture. The fresh unheated buffalo milk is placed in bamboo tubes covered with banana leaves, incubated at the ambient temperature (28–30 °C) overnight, and allowed to ferment naturally until it acquires a thick consistency (Akuzawa and Surono 2002).

#### 14.10.1.2 Important Lactic Acid Bacteria During Fermentation

The buffalo milk was poured into bamboo tubes and kept overnight at room temperature, stimulating the mesophilic indigenous LAB derived from the fresh raw milk to dominate and grow, allowing natural fermentation. Consequently, the fermentation of *dadih* is much longer than yogurt, 24 and 4 h, respectively, due to different types of LAB involved in the fermentation process at the incubation temperature, 28–30 °C and 45 °C, involving mesophilic cultures and thermophilic cultures, respectively, besides thicker consistency of *dadih*. Bamboo tube is hygroscopic and aided in keeping the product from wheying off.

The milk is fermented by indigenous LAB of the buffalo milk. Its natural fermentation provides different strains of indigenous lactic bacteria involved in each fermentation (Akuzawa and Surono 2002). The natural indigenous LAB observed in *dadih* could be derived from the bamboo tubes, buffalo milk, or banana leaves involved in milk fermentation, and buffalo milk has been observed to contribute the most, while bamboo tubes, banana leaves, and personal hygiene practice may also contribute.

Various indigenous lactic acid bacteria (LAB) involved in the *dadih* fermentation may vary from time to time, from one place to another due to the natural fermentation without any starter culture involved (Surono 2000; Akuzawa and Surono 2002). Interestingly, with minimum hygiene practice implemented, there was no product failure and no food poisoning reported among people consuming *dadih*. Instead, the older generation believes that consuming *dadih* may provide a beneficial effect to their health. Some *dadih* LAB have antimutagenicity, hypocholesterolemic properties, anti-pathogenic properties, and immunomodulatory properties (Surono and Hosono 1996a, b; Pato et al. 2004; Surono et al. 2011).

Hosono et al. (1989) reported that *Leu. paramesenteroides* predominates in *dadih* fermentation, responsible for producing aromatic compounds such as diacetyl, acetic acid, and other volatile compounds. Surono and Nurani (2001) found that *Lactobacillus* sp., *Lactococcus* sp., and *Leuconostoc* sp. were dominant in *dadih*. Surono (2003b) reported that among 20 colonies of *dadih* LAB isolated from Bukittinggi, West Sumatra, five strains were identified as *Lac. lactis* subsp. *lactis*, three strains of *Lb. brevis*, and three each of *Lb. plantarum*, *Lb. casei*, *Lb. paracasei*, and *Leu. mesenteroides*. Fresh *dadih* contains $4.3 \times 10^8$ cfu/g, dominated by lactic acid bacteria, which was $4.0 \times 10^8$ cfu/g. *Lc. cremoris*, *Lc. lactis*, *Lb. casei* subsp. *casei*, and *Lb. casei* subsp. *rhamnosus* were also found. Several strains belonging to *Ent. faecalis* subsp. *liquefaciens* were also found in *dadih*, indicating that the way of manufacturing dadih did not implement good hygiene practices, since microbes belong to the *Enterococci* group (Hosono et al. 1990). Surono and Nurani (2001) reported that the total viable lactic acid bacteria count was in the range of $1.42 \times 10^8$–$3.80 \times 10^8$ cfu/g in *dadih* originated from Bukittinggi and Padang Panjang area of West Sumatra.

Diverse microbes have been observed to involve in *dadih* fermentation due to traditional way of *dadih* manufacture. Surono et al. (1983) reported the involvement of yeast-like fungi at

$1.1 \times 10^7$ cfu/g, identified as *Endomyces lactis*, which is commonly found in dairy products. Imai et al. (1987) reported that the major bacterial species responsible for *dadih* fermentation were *Lb. casei* subsp. *casei* and *Lb. plantarum*. Microbial isolates of *dadih* have also been reported to exhibit probiotic attributes.

#### 14.10.1.3 Biochemical Changes During Buffalo Milk Fermentation

A consortium of LAB, which could be homofermentative and heterofermentative natural starter cultures producing lactic acid, with the involvement of beta-galactosidase from lactic starter cultures, results in coagulation of buffalo milk beginning at pH below 5.0 and completing at 4.6 (Surono (2003a). Texture, body, and acid flavor of *dadih* owe their origin to lactic acid produced during fermentation.

Small quantities of flavor compounds are generated through carbohydrate catabolism, via volatile fatty acids, ethanol, acetoin, acetic acid, butanone, diacetyl, and acetaldehyde. Homolactic starter cultures in *dadih* such as lactobacilli, lactococci, pediococci, and streptococci yield lactic acid as 95% of the fermentation output. Heterolactic starter cultures, such as *Lb. brevis*, *Lb. fermentum*, and *Leuconostoc* sp., contribute to flavor compounds. There are two important roles of lactic acid in *dadih* manufacture, which helps to destabilize the casein micelles and gives the *dadih* its distinctive and characteristic sharp acidic taste.

During fermentation, natural LAB multiply viable counts of $10^5$–$10^9$ cfu/g (Judoamidjojo et al. 1983; Hosono et al. 1989; Surono and Nurani 2001) and occupy about 1% volume of *dadih* product. These LAB cells contain cell walls, enzymes, nucleic acids, cellular proteins, lipids, and carbohydrates. Beta-galactosidase activity contributes a major conversion of lactose into LAB in *dadih*, which is beneficial for lactase-deficient people.

### 14.10.2 Probiotic Bacteria Isolated from *Dadih*

*Dadih* and several dairy products have been reported to consist of probiotic bacteria, which when consumed alive and in an adequate amount confer health benefit to the host (FAO/WHO 2002). The older generation believes that consuming *dadih* may provide a beneficial effect to their health. This fact has inspired more exploration of the powerful indigenous LAB involved during dadih fermentation, excluding the contaminants and the pathogens from the milk itself as well as environmental surroundings.

Collado et al. (2007a) reported that all the five strains of *dadih* origin showed good adhesion property, and the most adhesive was *Lb. plantarum* strain IS-10506. All LAB strains isolated from *dadih*-fermented milk were able to significantly reduce the adhesion levels of all the pathogens tested. *Lb. plantarum* IS-10506 and *Ent. faecium* IS-27526 had the highest inhibition abilities. The inhibitory, competitive, and displacing properties against pathogens were also observed. Hence, the two strains are promising candidates for future probiotics.

Furthermore, Surono et al. (2010) reported a significant increase of viable fecal LAB of rats after 3 days of administration with *Lb. plantarum* IS-10506 and *Lb. plantarum* IS-20506 at $1.2 \times 10^{10}$–$1.6 \times 10^{10}$ cfu/g each, by 3.25–3.5 and 0.35–0.65 log cycles, respectively, and continued the increment after 7 days, by 1.8–2.0 and 2.1–2.3 log cycles, respectively. The abilities of *dadih* LAB isolates in detoxifying mutagens (Hosono et al. 1990; Surono and Hosono 1996a, b) and cyanobacterial toxins have been reported. The mutagen absorbed and bound to the cell wall, while the cyanobacterial toxin was being metabolized (Surono et al. 2008, 2009; Nybom et al. 2008).

Many researchers reported hypocholesterolemic activity of *dadih*. Hosono and Tono-oka (1995) reported that *Lc. lactis* subsp. *lactis* biovar. *diacetylactis* R-43 and R-22 of dadih ori-

gin showed high-cholesterol-binding abilities, 33.91 % and 29.73 %, respectively. Surono (2003b) reported that *Lc. lactis* subsp. *lactis* strain IS-10285 and IS-29862 possess taurocholate-deconjugating abilities. Pato et al. (2004) found that rats fed with fermented milk made from *Lc. Lactis* subsp. *lactis* strain IS-10285 showed significant ($p<0.05$) lower total bile acids in serum. All these attributes show *dadih* as potential health-benefiting product. The probiotic properties of several strains isolated from *dadih* may provide the evidence on how strong the indigenous LAB derived from the fresh raw buffalo milk are in combating the contaminants, both spoilage bacteria and pathogens, during the spontaneous fermentation of *dadih* (Collado et al. 2007b).

Surono et al. (2011) reported significantly increased total salivary secretory IgA (sIgA) level and bodyweight of children ($p<0.05$) compared to placebo in a pilot randomized controlled trial on *Ent. faecium* IS-27526 isolated from *dadih* on children supplemented with lyophilized *Ent. faecium* IS-27526 ($2.31\times10^8$ cfu/g/) in 125 ml ultrahigh-temperature low-fat milk for 90 days. Changes of total salivary sIgA levels were significantly higher in underweight children supplemented with probiotic, while weight gain was observed significantly in children with normal bodyweight supplemented with probiotic.

In a 90-day randomized double-blind placebo-controlled pre-post trial , Surono et al. (2014) has been conducted on Indonesian children aged 12–24 months supplemented with microencapsulated *Lb. plantarum* IS-10506 of *dadih* origin, at $10^{10}$ cfu/g as probiotic, and 20 mg zinc sulfate monohydrate (8 mg zinc elemental) showed significant increase of fecal sIgA in probiotic group ($p<0.01$), and in probiotic and zinc group ($p<0.027$), as compared to placebo group. Changes of serum zinc concentration in the combination of probiotic and zinc group showed the highest elevation after supplementation. Supplementing probiotic *Lb. plantarum* IS-10506 and zinc for 90 days resulted in a significant increase of humoral immune response as well as improved zinc status of the young children (Surono et al. 2014).

## 14.11 Fermented Fish, Meat, and Egg Products

### 14.11.1 *Ikan Peda* (Fish Pickled with Salt)

Lightly salted fermented fish are mostly produced in Southeast Asia (Ishige 1993). Fish salted and packed in Thailand and then shipped to Malaysia and Indonesia accidentally underwent fermentation because the fish were still not completely dried. These fish developed a distinct flavor upon arriving in Indonesia and were named *pedah Siam* (Van Veen 1965).

*Ikan peda* (Fig. 14.17) is a wet fermented fish made from mackerel (*Scomber kanagurta*) or *Kembung* fish (*Rastrelliger neglectus*) mixed with 20–30 % salt fermented in two steps. In the primary fermentation, the eviscerated fish are fermented with high salt concentration (25 %, w/w) in a vessel and in sealed container for 3 days. The fish are then washed, drained, and piled in wooden boxes, putting banana leaves between the fish; then they are sprinkled with 30 % salt, covered with banana leaves, and fermented for another 1 week or longer, and an aroma characteristic of *ikan peda* is developed. Then the *ikan peda* is dried in the air (Putro 1993).

Rahayu (2003) isolated *Lb. plantarum*, *Lb. curvatus*, *Lb. murinus*, and *Strep. thermophilus*

**Fig. 14.17** *Ikan peda*

from *ikan peda*. During fermentation, enzymes derived from the fish and halophilic bacteria, including lactic acid bacteria, are involved which produce lactic acid and preserved the fish. According to Van Veen (1965), the best *ikan peda* has moisture content of 44–47 %, 7–14 % fat, 21–22 % protein, and 15–17 % NaCl.

*Ikan peda* is described as being fatty, partly dried salty fish with reddish brown color, moist and slightly pasty with a flabby texture, and a specific flavor, which is cheesy, tasty, salty, and often mixed with mild rancid flavor. Prolonged storage during retailing will facilitate the development of rancid flavor accompanied by a change in meat color to reddish brown (Van Veen 1965; Hanafiah 1987).

## 14.11.2 *Terasi* (Shrimp Paste)

*Terasi* is one of the salty fermented products that undergo alkaline fermentation, made from fish and/or shrimp which is in the form of paste (Fig. 14.18). *Terasi* and *tauco* serve similar roles as condiments due to the presence of glutamic acid and its specific flavor.

*Terasi* is an indigenous fermented food in Indonesia, salted fish or shrimp, mixed with 2–5 % salt, dried repeatedly, ground into a fine paste, and allowed to ferment naturally for a period of several weeks until the desired flavor has developed, followed by sun-drying for 1–3 days. It has a flavor reminiscence of ripe cheese. Closely related products are the Filipino *Bagoong*, Burmese *Ngapi*, Malaysian *Belacan*, and Thai *Kappi*. The most important center of its manufacture is Bagansiapiapi, North Sumatra (Surono and Hosono 1994a).

### 14.11.2.1 Manufacturing *Terasi*

*Terasi* in general is prepared as follows: The shrimp or fish are mixed with salt at10 % level on the fishing boats and then spread out on the floor. Further salt is added at 5 %, and the product is dried in the sun for about 1–3 days, occasionally turned over to decrease the moisture content from about 80 % to 50 % and also to minimize off flavor. The resulting mass is minced, pressed tightly into wooden tubs to exclude the air and allow to ferment 1–4 weeks (Surono and Hosono 1994a).

### 14.11.2.2 Chemical Composition of *Terasi*

The protein content of *terasi* was 25.42 g/100 g, with glutamic acid as dominant amino acid, 17.73 g/100 g. The pH of *terasi* was 7.53, with a salt content of 16.75 g/100 g, a fat content of 6.11 g/100 g, and a carbohydrate content of 1.94 g/100 g. Its moisture content was 37.41 % (Surono and Hosono 1994a).

### 14.11.2.3 Microbial and Biochemical Changes of *Terasi*

Surono and Hosono (1994a) reported that acid-producing *Bacillus* sp. and *Pseudomonas* sp. were dominating the microbial population involved during *terasi* fermentation. On the other hand, *Kurthia gibsonii* and *Sporolactobacillus*

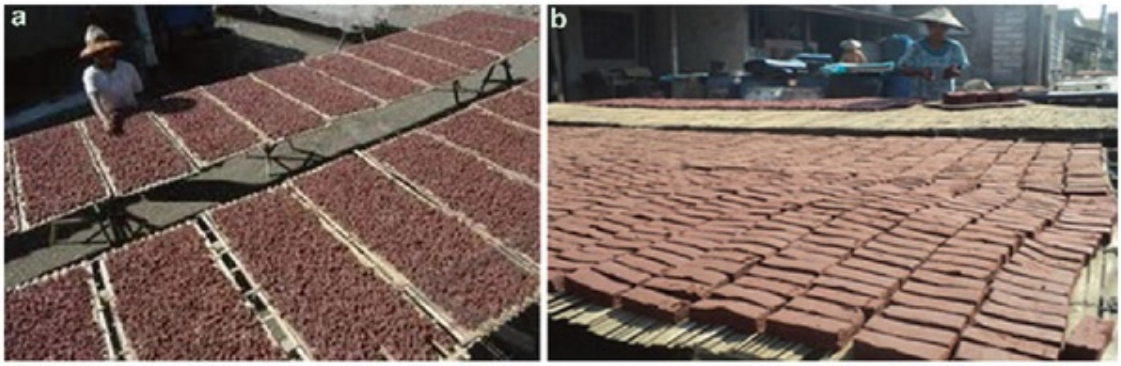


**Fig. 14.18** (**a**) *Terasi* fermentation, (**b**) *Terasi* in block

*inulinus* represented minority of aerobic bacteria population in terasi, while *Micrococcus* sp. had the ability to utilize protein but poor in utilizing carbohydrate.

According to Surono and Hosono (1994a), during the early stage of *terasi* fermentation, the pH drops to 4.5 and most *Pseudomonas* sp. failed to grow. Since *terasi* are manufactured without any concern on good hygiene practice, *Pseudomonas* sp. may represent contaminants during mixing, drying, and packaging of terasi. This kind of bacteria grows well in proteinaceous food and actively spoils seafoods.

In the presence of 10 % salt, during fermentation, the species *Bacillus*, *Pediococcus*, *Lactobacillus*, *Micrococcus*, *Sarcina*, *Staphylococcus*, *Clostridium*, *Brevibacterium*, *Flavobacterium*, and *Corynebacterium* were decreased. At the beginning of terasi fermentation, there was an increase in total bacteria dominated by lactic acid bacteria, micrococci, and bacilli, which decrease at the end of fermentation. The endogenous enzymes from *B. subtilis* and *B. coagulans* together with the enzymes derived from intestines of the fish hydrolyzed the protein (Surono and Hosono 1994b). The bacterial enzymes were mainly responsible for the deamination and decarboxylation of amino acids to form lower fatty acids and amides, producing characteristic flavor of *terasi*. The combination of salt and the microbial degradation products of protein, fat, and carbohydrate may contribute to the taste and aroma of the *terasi* (Aryanta 2000).

The endogenous proteolytic enzymes, rather than bacteria, are responsible for the hydrolysis of fish muscle, prior to the bacterial activity. Furthermore, the halophiles could possibly hydrolyze short-chain fatty acids; hence, the presence of esterase (C4) and esterase lipase (C8) activities in all of the bacteria presented in *terasi* starter supports the hypothesis that bacterial enzymes hydrolyze the fat, producing low-molecular-weight fatty acids that are responsible for the cheesy odor Surono and Hosono (1994b).

The total halophilic count of *terasi* was $1.1 \times 10^5$ cfu/g, dominated by halophilic *Bacillus* sp. *B. pumilus* is the dominant species throughout the fermentation. Other bacteria responsible for early stage of fermentation were *B. coagulans*, *B. megaterium*, *and B. subtilis*, while in the later stage of fermentation, *B. licheniformis*, *M. colpogenes*, *M. roseus*, *M. varians*, and *Staphylococcus* sp. were found in *terasi* (Surono and Hosono 1994a).

Surono and Hosono (1994a) reported that *terasi* starter was composed of *B. brevis*, *B. pumilus*, *B. megaterium*, *B. coagulans*, *B. subtilis*, and *M. kristinae* in the proportion of 39.1 %, 26.1 %, 8.7 %, 8.7 %, 8.7 %, and 8.7 %, respectively. All of the microflora found in *terasi starter* were salt tolerant, as shown by their capability to grow on agar plates in the presence of 10 % NaCl. The dominant flora were *Bacillus* sp., which are halophilic and aerobic, grow in the temperature range of 10–50 °C, and have esterase (C4) and esterase lipase (C8) activities. None of the isolates had the ability to produce gas from glucose, even though most of the isolates could produce acid from glucose (Surono and Hosono 1994a). Only *B. pumilus*, *B. coagulans*, and *M. kristinae* had the ability to hydrolyze long-chain fatty acids. *Terasi* has a typically characteristic aroma of cheese and ammonia. The cheesy odor is produced by low-molecular-weight fatty acids, and the ammoniacal odor is due to the presence of amines and ammonia (Dougan and Howard 1975).

*T. muriaticus* strain was found and reported to produce histamine in fermented fish in Japan and Thailand. Viable cell lactic acid bacterial counts in *terasi* were $10^4$–$10^6$ cfu/g. All the isolates were catalase negative, were Gram-positive cocci, and were able to grow at 15 % NaCl and classified into two types: the *Tetragenococcus halophilus* group and the *T. muriaticus* group as revealed by a restriction fragment length polymorphism (RFLP) analysis and sequencing of the 16S rRNA gene (Kobayashi et al. 2000, 2003).

### 14.11.3 *Urutan* (Traditional Balinese Pork Sausage)

*Urutan* (Fig. 14.19) is usually prepared to celebrate the Galungan Day, which is a special holy day for Hindus in Bali. A day before the feast, Balinese people slaughter pigs to prepare tradi-

**Fig. 14.19** *Urutan*, fermented pork sausage (Picture courtesy of Prof. Nyoman Semadi Antara)

tional foods. Due to the excessive fresh pork available, it needs to be preserved to prolong the shelf life of the meat which can then be consumed on the following days or weeks. Two common Balinese practices to preserve the pork meat are by drying *dendeng* and fermenting *urutan*.

*Urutan* is a Balinese traditional fermented sausage, which is made of chopped lean pork and fat which are mixed with spices (garlic, turmeric, aromatic ginger, chili, and pepper), sugar, and salt. The mixture is filled into cleaned pig intestine and fermented spontaneously and then sun-dried for 2–5 days (Aryanta 2000).

*Urutan* has a different microbial ecology, compared with other fermented sausages, and this is primarily due to spontaneous fermentation which occurs during the drying process, and the quality of the product varies from time to time, from place to place. The use of spices and high-temperature fermentation is of special interest in relation to the characteristics of lactic acid bacteria involved in the process, and their distribution plays an important role in food fermentation, responsible for the characteristic flavor development, and contributes to the preservation of the fermented product. The pork meat inside the casing became more compact and solid after fermentation. Traditionally, *urutan* was manufactured without any addition of nitrite and/or nitrate, and the color became dark red at the end of fermentation. The products were dried under the sun, and the juice (mixture of oil and water) was dripped so that the casing becomes dry and wrinkled (Antara et al. 2002).

#### 14.11.3.1 Microbial and Biochemical Changes During Urutan Fermentation

The total viable lactic acid bacterial counts were remarkably increased from the initial count of $1.72 \times 10^5$–$1.84 \times 10^8$ cfu/g on day 1 and then decreased to $4.98 \times 10^7$ cfu/g at days 2–5 (Antara et al. 2002). The results also showed that lactic acid bacteria predominated the total microbial population. The sharp reduction of pH on the first day of fermentation affected the growth of Enterobacteriaceae, which were not detected at day 2 of fermentation. Soluble protein decreased at the first day of fermentation. Total acidity increased significantly after day 1 of fermentation, and it remained constant until the end of the process. The carbon sources in the product primarily sugar and spices were utilized by homofermentative lactic acid bacteria and converted into lactic acid as the lactic acid bacteria grow and multiply during fermentation. There was no butyric acid detected, and only small amounts of succinic and propionic acids were produced (Antara et al. 2004).

Antara et al. (2002) found that in *urutan*, 77.5 % of bacteria are lactobacilli, and the other 22.5 % are pediococci. Further molecular identification by 16S rDNA sequence revealed that *Lb. plantarum*, *Lb. farciminis*, and obligate heterofermentative lactobacilli *Lb. fermentum* and *Lb. hilgardii*. Besides, *P. acidilactici* and *P. pentosaceus* were also detected (Antara et al. 2002). Microbial succession occurred during fermentation of *urutan*, dominated at initial growth by *Lb. plantarum*, predominated during 2 days of fermentation, then followed by *P. acidilactici*, and finally, *Lb.*

*farciminis* was found to be predominant at the last stage of fermentation (Antara et al. 2004).

*P. acidilactici* was found after 2 days and slightly decreased until the end of fermentation. On the other hand, *Lb. farciminis* was distributed in an increasing manner from day 2 until the end of fermentation process. The heterofermentative lactobacilli (*Lb. fermentum* and *Lb. hilgardii*) were not significantly detected during the whole fermentation, only detected in small number on the third and fifth days, respectively (Antara et al. 2004). Most of lactic acid bacteria present in *urutan* during fermentation were identified as the homofermentative type, which produced mostly lactic acid and a small amount of acetic acid as well as carbon dioxide (Aryanta 1998).

The rapid growth of LAB during the first day of fermentation of urutan is in agreement with those reported for salami; the LAB in this product decreased after 2 days of fermentation and remained constant until the end of the process (Antara et al. 2002), in contrast to the LAB growth in salami whereby the LAB population starts to increase on the first day, reaching a maximum of 7 days, and remains constant until the end of the ripening period (Coppola et al. 1998, 2000). The difference of LAB growth in urutan might be due to the use of local types of spices, especially aromatic ginger and turmeric, and the high concentration of garlic suppressed the growth of LAB in *urutan* (Antara et al. 2002).

*Lb. plantarum* dominating the total lactic acid bacterial population during fermentation makes *urutan* different from salami where *Lb. sake* and *Lb. curvatus* were present as the dominant species (Coppola et al. 2000). The presence of *P. acidilactici* in *urutan*, which was also distributed significantly, could be due to the high fermentation temperature. This species is widely used for rapid fermentation with temperature above 30 °C (Ordonez et al. 1999). The existence of *P. acidilactici* as bacteriocin producer plays an important role for the succession. The bacteriocin produced in this product inhibited the growth of *Lb. plantarum and P. pentosaceus*, but the growth of *Lb. farciminis* was stimulated by the intrinsic condition of urutan after 3 days until the end of fermentation.

The heterofermentative lactobacilli were only existent at the final stage of fermentation as shown by the lactic acid produced, and the absence of gas in *urutan* indicated that the role of the heterofermentative lactobacilli was not significant since, as reported, the heterofermentative lactobacilli (*Lb. fermentum* and *Lb. hilgardii*) were not significantly detected during the whole fermentation. The existence of these species was only in small number on the third and fifth days, respectively (Antara et al. 2002).

The denaturation of protein and the absence of bubbles from $CO_2$ made the texture of *urutan* more compact and solid (Antara et al. 2002). Inconsistent of quality and risk of fermentation failure are the common problems on traditional fermented products which are produced under spontaneous natural fermentation indicating off-odor development. Antara et al. (2004) reported that at pH 4.3–4.5, urutan (Balinese dry fermented sausage) using mixed LAB as the starter culture might inhibit the growth of Clostridia.

#### 14.11.3.2 *Telur Asin* (Salted egg)

*Telur asin* (salted egg) is an alkaline-fermented ethnic food in Indonesia (Fig. 14.20). Alkaline-fermented foods constitute a group of less-known food products that are widely consumed in Southeast Asia and African countries. Traditional processing of *telur asin* is carried out by coating method. The fresh duck eggs are coated with a muddy paste containing ash or red brick powder and salt at 1:1, allowed to ferment in a jar for 15–20 days, and the telur asin can be preserved for 2–3 weeks (Margono et al. 2000). Suprapti (2002) reported that the use of ash as coating agent will produce *telur asin* with pale yellow and grayish surrounding the yolk, while the use of red brick powder produces *telur asin* with reddish yolk.

In alkaline-fermented foods, the protein of the raw materials is broken down into amino acids and peptides; ammonia is released during the fermentation, raising the pH of the final products and giving the food a strong ammoniacal smell in spontaneous fermentation by mixed bacteria cultures.

**Fig. 14.20** Boiled *telur asin*, *telur asin* covered with muddy ash and salt, smoked *telur asin*

Saputra (2013) reported that *Lb. plantarum*, *Lb. casei* subsp. *rhamnosus*, *Enterococcus gallinarum*, and *P. acidilactici* have been isolated from *telur asin* and inhibited the pathogen, especially *E. coli and Staph aureus by organic acids produced and* bacteriocin produced by *P. acidilactici*. *Telur asin* may also be smoked, producing rich unique flavor of salty and smoked taste.

## 14.12 Fermented Roots and Tuber Products

### 14.12.1 *Tapé*, *Growol*, and *Gatot*

*Tapé* is a sweet and sour fermented food with an alcoholic flavor, prepared from glutinous rice or cassava or other cereals by using *ragi starter* in Indonesia (Campbell-Platt 1994). It is eaten as dessert or delicacy in Indonesia. There are various starchy substrates used to prepare *tapé*, such as cassava (*tapé singkong*), glutinous rice (*tapé ketan*), maize (*tapé jagung*), and millet (*tapé cantel*). The raw material is washed, soaked, steamed, cooled to room temperature on a woven bamboo tray, sprinkled with *ragi* powder, packed in small banana leaves, and fermented for 2–3 days at room temperature, and a soft juicy mass of *tapé* is produced (Saono et al. 1977). *Tapé singkong* is deep-fried in coconut oil and baked before consumption. The alcohol content ranged from 3% to 8.5% v/v (Cronk et al. 1977). The glutinous rice lipids are hydrolyzed during *tapé ketan* fermentation (Cronk et al. 1979).

*Tape* is a traditional fermented food in Indonesia and also in Malaysia, the Philippines, and Vietnam, made from raw material containing starch and *ragi*, the microbial starter. *Ragi* is a mixture of rice flour, spices, sugar cage, water, and yeasts and used as inoculums in alcoholic fermentation in natural fermentation. Aryanta (1988) isolated *Rz. oryzae*, *M. rouxii*, *Asp. oryzae*, *S. cerevisiae*, *E. burtonii*, *H. anomala*, and *P. pentosaceus* from *ragi* (NKL brand) obtained from Denpasar. On the other hand, Winarni (1988) isolated some amylolytic molds and yeasts from four types of local commercial *ragi*. *P. pentosaceus* is the acid producer commonly found in ragi and most abundantly isolated from newly collected ragi samples in Indonesia, followed by a small number of *Lb. plantarum* and *Lb. fermentum* strains (Uchimura et al. 1998).

*Tape singkong* (Fig. 14.21) is a fermented steam cassava by *ragi tape* starter, involving *Amylomyces rouxii* and *E. burtonii*, *S. cerevisiae*, *Rhizopus* sp., and *Hansenula* sp. (Steinkraus 1983; Hassan et al. 1986; Suliantari and Rahayu 1990).

**Fig. 14.21** *Tape singkong* (fermented cassava)

The fungi *Rz. arrhizus* var. *rouxii* strain TT is able to utilize starch and convert it to alcohol, producing soft, juicy, sweet tape which lack sourness, and 1.88 % (w/v) ethanol was produced after 72 h of fermentation (Hassan et al. 1986). The yeast is inoculated together with the fungi to be able to do the fermentation, while inoculating yeast only, the fermentation does not occur.

#### 14.12.1.1 Biochemical Changes

The fermentation process of *tape singkong* has two main stages: the conversion of starch into simple sugars carried out by amylase producers (molds and yeasts) and the conversion of sugars into alcohol and acids carried out by certain yeasts.

Esters are also formed due to the reaction between alcohol and acids which contribute to the sweet–sour taste with a mild alcoholic flavor of the product (Djien 1972; Karim and Hassan 1986). The pH of *tape singkong* decreased from an initial value of 5.65–5.15 during the first 4 days of fermentation, while total acidity increased from 3.5 mg/100 g to 5.2 mg/100 g. During the first 4 days of fermentation, reducing sugar increased from 7.9 % to 16.0 % and decreased to 12.73 % after this time (Rahayu 1980).

*Tape singkong* is sweet, slightly sour, and aromatic, but too much acid content in *tape* is undesirable. The nutrient contents of *tape singkong* are as follows: 0.5 % protein, 0.1 % fat, 42.5 % carbohydrate, and 56 % moisture (Winarno 1983).

Mixed cultures of *Streptococcus*, *Rhizopus*, and *Saccharomycopsis* produce aroma in *tapé*, whereas *Sm. fibuligera* produces α-amylase and *Rhizopus* sp. produces glucoamylase (Suprianto et al. 1989).

*Growol* is fermented raw cassava tubers, whereas *gatot* is fermented dried cassava tubers involving lactic acid bacteria. These products are popular in certain part of Java. The manufacture of fermented tubers is by soaking the peeled raw cassava for growol and dried cassava for gatot for several days until the tubers become soft.

*Growol* is a traditional fermented food made from cassava which has sour taste, only found in Yogyakarta area, especially Kulon Progo and the surrounding area. The peeled and sliced cassava was soaked in the water for 4 days, drained, and crushed before being steamed.

The fermentation occurs naturally; *Coryneform*, *Streptococcus*, *Bacillus*, and *Actinobacteria* grow at the beginning of the fermentation followed by *Lactobacillus* and yeast until the end of fermentation. Suharni (1984) reported that lactic acid bacteria dominated and found at $1.64 \times 10^8$ cfu/g. *Lactobacillus casei* subsp. *rhamnosus* TGR2 isolated from *growol* produces extracellular metabolites which remain stable at room temperature and has resistance to heating at 98 °C for 30 min, at pH 3–8 (Rahayu 1995).

Gatot, dried cassava, is a traditional fermented food in Gunung Kidul, Yogyakarta. Cassava is peeled dried to get the typical black color of *gatot* as a staple food. Ichsyani (2014) reported that *P. pentosaceus* and *Saccharomyces* sp. *TR7* isolated from *gatot* have amylolytic activity and able to reduce cyanide in cassava. *Lb. plantarum* 250 Mut7 FNCC also has been isolated from *gatot* (Harmayani et al. 2001) and showed the ability in suppressing bacteroides (Sari 2014).

## 14.13 Conclusion

Traditional fermented foods are mostly carried out involving mixed cultures. The preparation and consumption of traditional foods are the traditional wisdom of the people in each area, which have fostered a distinct food culture of the people. Fermented foods are known to be not only

nutritious but also healthy foods. The Indonesian biodiversity of microbes involved in the traditional fermentation which may vary from time to time, from place to place, may give opportunity to the exploration of the beneficial effects of the microbes, enzymes involved, and the metabolites as functional foods, such as probiotics, some enzymes in combating the metabolic syndrome or degenerative diseases, and even for producing ethanol as biofuel to support human welfare.

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